# PYRROLOBENZODIAZEPINES

The present invention relates to pyrrolobenzodiazepines (PBDs),
and in particular pyrrolobenzodiazepines having a C2-C3 double
5   bond, an aryl group at the C2 position, and particular
substituents on that C2 aryl group.

Background to the invention

Some pyrrolobenzodiazepines (PBDs) have the ability to recognise
10 and bond to specific sequences of DNA; the preferred sequence is
PuGPu. The first PBD antitumour antibiotic, anthramycin, was
discovered in 1965 (Leimgruber, et al., J. Am. Chem. Soc., **87**,
5793-5795 (1965); Leimgruber, et al., J. Am. Chem. Soc., **87**,
5791-5793 (1965)). Since then, a number of naturally occurring
15 PBDs have been reported, and over 10 synthetic routes have been
developed to a variety of analogues (Thurston, et al., Chem. Rev.
1994, 433-465 (1994)). Family members include abbeymycin
(Hochlowski, et al., J. Antibiotics, **40**, 145-148 (1987)),
chicamycin (Konishi, et al., J. Antibiotics, **37**, 200-206 (1984)),
20 DC-81 (Japanese Patent 58-180 487; Thurston, et al., Chem. Brit.,
26, 767-772 (1990); Bose, et al., Tetrahedron, **48**, 751-758
(1992)), mazethramycin (Kuminoto, et al., J. Antibiotics, **33**,
665-667 (1980)), neothramycins A and B (Takeuchi, et al., J.
Antibiotics, **29**, 93-96 (1976)), porothramycin (Tsunakawa, et al.,
25 J. Antibiotics, **41**, 1366-1373 (1988)), prothracarcin (Shimizu, et
al, J. Antibiotics, **29**, 2492-2503 (1982); Langley and Thurston,
J. Org. Chem., **52**, 91-97 (1987)), sibanomicin (DC-102)(Hara, et
al., J. Antibiotics, **41**, 702-704 (1988); Itoh, et al., J.
Antibiotics, **41**, 1281-1284 (1988)), sibiromycin (Leber, et al.,
30 J. Am. Chem. Soc., **110**, 2992-2993 (1988)) and tomamycin (Arima,
et al., J. Antibiotics, **25**, 437-444 (1972)). PBDs are of the
general structure:

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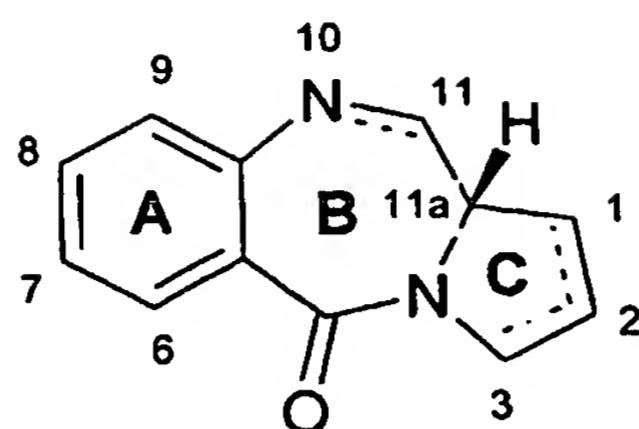
They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is

5 either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe)) at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (S)-configuration at the chiral C11a position which provides them with a right-handed

10 twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In *Antibiotics III.* Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, *Acc. Chem.*

15 *Res.*, **19**, 230-237 (1986)). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, hence their use as antitumour agents.

The present inventors have previously disclosed, in WO 00/12508,

20 that the following compounds, amongst many other, are cytotoxic:
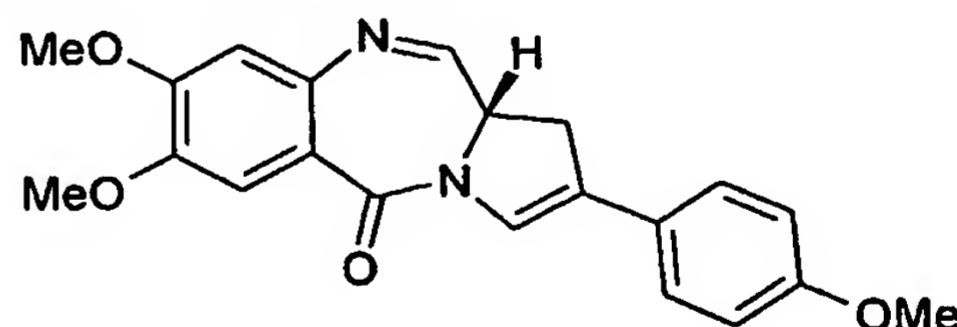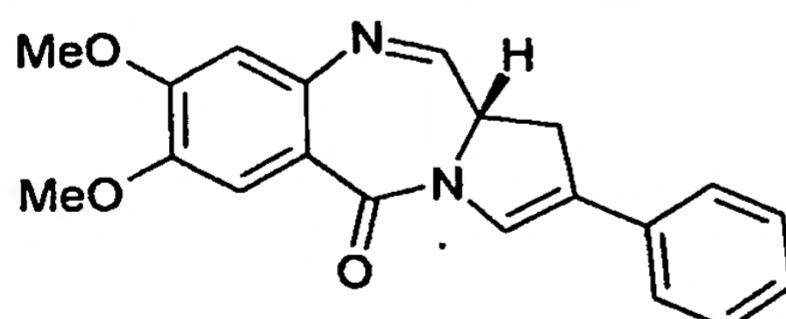
More recently, in Cooper. N., *et al.*, *Chem. Commun.*, **16**, 1764-1765 (2002), the following compound was also disclosed as being

25 cytotoxic alongside the compounds shown above.

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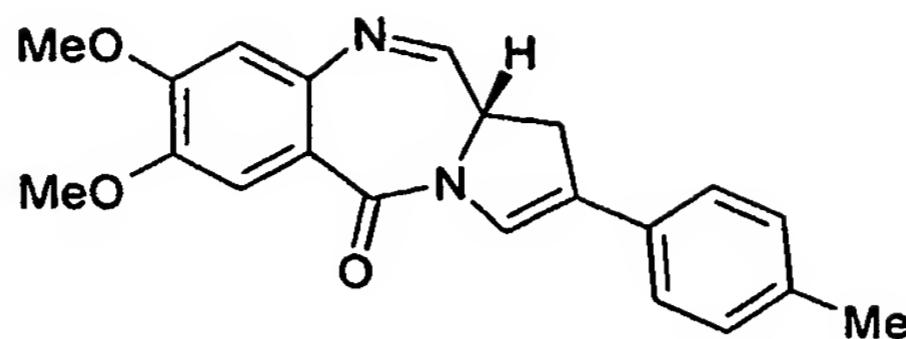
Disclosure of the invention

The present inventors have now discovered that a certain class of
5   these compounds exhibit surprising activity against cancer cell
lines, compared to the known compounds discussed above.

Therefore, a first aspect of the present invention is a compound
of formula (I):

(I)
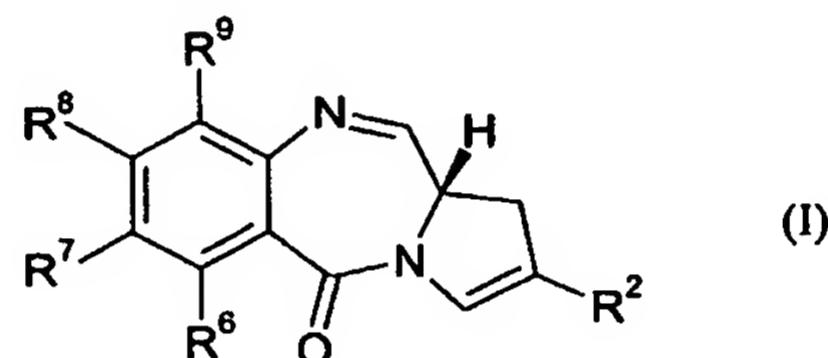
10
and salts, solvates, chemically protected forms, and prodrugs
thereof, wherein:

R^6, R^7 and R^9 are independently selected from H, R, OH, OR, SH,
SR, NH_2, NHR, NHRR', nitro, Me_3Sn and halo;

15 where R and R' are independently selected from optionally
substituted C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;
R^8 is selected from H, R, OH, OR, SH, SR, NH_2, NHR, NHRR', nitro,
Me_3Sn and halo, or the compound is a dimer with each monomer being
of formula (I), where the R^8 groups of each monomers form together

20 a dimer bridge having the formula -X-R"-X- linking the monomers,
where R" is a C_{3-12} alkylene group, which chain may be interrupted
by one or more heteroatoms, e.g. O, S, NH, and/or aromatic rings,
e.g. benzene or pyridine, and each X is independently selected
from O, S, or NH;

25 or any pair of adjacent groups from R^6 to R^9 together form a group
$-O-(CH_2)_p-O-$, where p is 1 or 2; and
R^2 is selected from:
(i)   an optionally substituted napthyl group;
(ii)   an optionally substituted thiophenyl or furanyl group; and

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(iii) a phenyl group substituted by:

        (a)   one or more chloro or fluoro groups;

        (b)   an ethyl or propyl group;

        (c)   a 4-t-butyl group;

5   (d)   a 2-methyl group;

        (e)   two methyl groups in the 2- and 6- positions.

A second aspect of the present invention is the use of a compound of the first aspect of the invention in a method of therapy.

10

A third aspect of the present invention is a pharmaceutical composition containing a compound of the first aspect of the invention, and a pharmaceutically acceptable carrier or diluent.

15 A fourth aspect of the present invention provides the use of a compound of the first aspect of the invention in the manufacture of a medicament for treating a proliferative disease.

One of ordinary skill in the art is readily able to determine
20 whether or not a candidate compound treats a proliferative condition for any particular cell type. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described in the examples below.

25 The term "proliferative disease" pertains to an unwanted or uncontrolled cellular proliferation of excessive or abnormal cells which is undesired, such as, neoplastic or hyperplastic growth, whether *in vitro* or *in vivo*.

30 Examples of proliferative conditions include, but are not limited to, benign, pre-malignant, and malignant cellular proliferation, including but not limited to, neoplasms and tumours (e.g. histocytoma, glioma, astrocyoma, osteoma), cancers (e.g. lung cancer, small cell lung cancer, gastrointestinal cancer, bowel
35 cancer, colon cancer, breast carinoma, ovarian carcinoma, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreas cancer, brain cancer, sarcoma,

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osteosarcoma, Kaposi's sarcoma, melanoma), leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis.

5   Any type of cell may be treated, including but not limited to, lung, gastrointestinal (including, e.g. bowel, colon), breast (mammary), ovarian, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

10   Substituents

The phrase "optionally substituted" as used herein, pertains to a parent group which may be unsubstituted or which may be substituted.

15   Unless otherwise specified, the term "substituted" as used herein, pertains to a parent group which bears one or more substitutents. The term "substituent" is used herein in the conventional sense and refers to a chemical moiety which is covalently attached to, or if appropriate, fused to, a parent

20   group. A wide variety of substituents are well known, and methods for their formation and introduction into a variety of parent groups are also well known.

Examples of substituents are described in more detail below.

25

C_{1-7} alkyl: The term "C_{1-7} alkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 7 carbon atoms, which may be aliphatic or alicyclic, and which may be

30   saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). Thus, the term "alkyl" includes the sub-classes alkenyl, alkynyl, cycloalkyl, etc., discussed below.

Examples of saturated alkyl groups include, but are not limited

35   to, methyl (C_1), ethyl (C_2), propyl (C_3), butyl (C_4), pentyl (C_5), hexyl (C_6) and heptyl (C_7).

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Examples of saturated linear alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), n-propyl (C_3), n-butyl (C_4), n-pentyl (amyl) (C_5), n-hexyl (C_6) and n-heptyl (C_7).

5   Examples of saturated branched alkyl groups include iso-propyl (C_3), iso-butyl (C_4), sec-butyl (C_4), tert-butyl (C_4), iso-pentyl (C_5), and neo-pentyl (C_5).

C_{2-7} Alkenyl: The term "C_{2-7} alkenyl" as used herein, pertains to
10   an alkyl group having one or more carbon-carbon double bonds.

Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, $-CH=CH_2$), 1-propenyl ($-CH=CH-CH_3$), 2-propenyl (allyl, $-CH-CH=CH_2$), isopropenyl (1-methylvinyl,
15   $-C(CH_3)=CH_2$), butenyl (C_4), pentenyl (C_5), and hexenyl (C_6).

C_{2-7} alkynyl: The term "C_{2-7} alkynyl" as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds.

20   Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl (ethinyl, $-C\equiv CH$) and 2-propynyl (propargyl, $-CH_2-C\equiv CH$).

C_{3-7} cycloalkyl: The term "C_{3-7} cycloalkyl" as used herein,
25   pertains to an alkyl group which is also a cyclyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a cyclic hydrocarbon (carbocyclic) compound, which moiety has from 3 to 7 carbon atoms, including from 3 to 7 ring atoms.
30
Examples of cycloalkyl groups include, but are not limited to, those derived from:
      saturated monocyclic hydrocarbon compounds:
cyclopropane (C_3), cyclobutane (C_4), cyclopentane (C_5),
35   cyclohexane (C_6), cycloheptane (C_7), methylcyclopropane (C_4), dimethylcyclopropane (C_5), methylcyclobutane (C_5), dimethylcyclobutane (C_6), methylcyclopentane (C_6),

dimethylcyclopentane (C_7) and methylcyclohexane (C_7);

　　　unsaturated monocyclic hydrocarbon compounds: cyclopropene (C_3), cyclobutene (C_4), cyclopentene (C_5), cyclohexene (C_6), methylcyclopropene (C_4), dimethylcyclopropene (C_5), methylcyclobutene (C_5), dimethylcyclobutene (C_6), methylcyclopentene (C_6), dimethylcyclopentene (C_7) and methylcyclohexene (C_7); and

　　　saturated polycyclic hydrocarbon compounds: norcarane (C_7), norpinane (C_7), norbornane (C_7).

C_{3-20} heterocyclyl: The term "C_{3-20} heterocyclyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which moiety has from 3 to 20 ring atoms, of which from 1 to 10 are ring heteroatoms. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

In this context, the prefixes (e.g. C_{3-20}, C_{3-7}, C_{5-6}, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "C_{5-6}heterocyclyl", as used herein, pertains to a heterocyclyl group having 5 or 6 ring atoms.

Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from:

N_1: aziridine (C_3), azetidine (C_4), pyrrolidine (tetrahydropyrrole) (C_5), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C_5), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C_5), piperidine (C_6), dihydropyridine (C_6), tetrahydropyridine (C_6), azepine (C_7);

O_1: oxirane (C_3), oxetane (C_4), oxolane (tetrahydrofuran) (C_5), oxole (dihydrofuran) (C_5), oxane (tetrahydropyran) (C_6), dihydropyran (C_6), pyran (C_6), oxepin (C_7);

S_1: thiirane (C_3), thietane (C_4), thiolane (tetrahydrothiophene) (C_5), thiane (tetrahydrothiopyran) (C_6), thiepane (C_7);

O_2: dioxolane (C_5), dioxane (C_6), and dioxepane (C_7);

O_3: trioxane (C_6);

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N_2: imidazolidine (C_5), pyrazolidine (diazolidine) (C_5), imidazoline (C_5), pyrazoline (dihydropyrazole) (C_5), piperazine (C_6);

N_1O_1: tetrahydrooxazole (C_5), dihydrooxazole (C_5),

5   tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);

N_1S_1: thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);

N_2O_1: oxadiazine (C_6);

O_1S_1: oxathiole (C_5) and oxathiane (thioxane) (C_6); and,

10 $N_1O_1S_1$: oxathiazine (C_6).

Examples of substituted monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, furanoses (C_5), such as arabinofuranose, lyxofuranose,

15   ribofuranose, and xylofuranse, and pyranoses (C_6), such as allopyranose, altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

C_{5-20} aryl: The term "C_{5-20} aryl", as used herein, pertains to a

20 monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 3 to 20 ring atoms. Preferably, each ring has from 5 to 7 ring atoms.

25 In this context, the prefixes (e.g. C_{3-20}, C_{5-7}, C_{5-6}, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "C_{5-6} aryl" as used herein, pertains to an aryl group having 5 or 6 ring atoms.

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The ring atoms may be all carbon atoms, as in "carboaryl groups". Examples of carboaryl groups include, but are not limited to, those derived from benzene (i.e. phenyl) (C_6), naphthalene (C_{10}), azulene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), naphthacene

35 (C_{18}), and pyrene (C_{16}).

Examples of aryl groups which comprise fused rings, at least one of which is an aromatic ring, include, but are not limited to, groups derived from indane (e.g. 2,3-dihydro-1H-indene) (C_9), indene (C_9), isoindene (C_9), tetraline
5 (1,2,3,4-tetrahydronaphthalene (C_{10}), acenaphthene (C_{12}), fluorene (C_{13}), phenalene (C_{13}), acephenanthrene (C_{15}), and aceanthrene (C_{16}).

Alternatively, the ring atoms may include one or more
10 heteroatoms, as in "heteroaryl groups". Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:

N_1: pyrrole (azole) (C_5), pyridine (azine) (C_6);

O_1: furan (oxole) (C_5);
15 S_1: thiophene (thiole) (C_5);

N_1O_1: oxazole (C_5), isoxazole (C_5), isoxazine (C_6);

N_2O_1: oxadiazole (furazan) (C_5);

N_3O_1: oxatriazole (C_5);

N_1S_1: thiazole (C_5), isothiazole (C_5);
20 N_2: imidazole (1,3-diazole) (C_5), pyrazole (1,2-diazole) (C_5), pyridazine (1,2-diazine) (C_6), pyrimidine (1,3-diazine) (C_6) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) (C_6);

N_3: triazole (C_5), triazine (C_6); and,

N_4: tetrazole (C_5).
25
Examples of heteroaryl which comprise fused rings, include, but are not limited to:

C$_9$ (with 2 fused rings) derived from benzofuran (O_1), isobenzofuran (O_1), indole (N_1), isoindole (N_1), indolizine (N_1),
30 indoline (N_1), isoindoline (N_1), purine (N_4) (e.g., adenine, guanine), benzimidazole (N_2), indazole (N_2), benzoxazole (N_1O_1), benzisoxazole (N_1O_1), benzodioxole (O_2), benzofurazan (N_2O_1), benzotriazole (N_3), benzothiofuran (S_1), benzothiazole (N_1S_1), benzothiadiazole (N_2S);
35 C$_{10}$ (with 2 fused rings) derived from chromene (O_1), isochromene (O_1), chroman (O_1), isochroman (O_1), benzodioxan (O_2), quinoline (N_1), isoquinoline (N_1), quinolizine (N_1), benzoxazine

(N_1O_1), benzodiazine (N_2), pyridopyridine (N_2), quinoxaline (N_2), quinazoline (N_2), cinnoline (N_2), phthalazine (N_2), naphthyridine (N_2), pteridine (N_4);

C_{11} (with 2 fused rings) derived from benzodiazepine (N_2);

5   C_{13} (with 3 fused rings) derived from carbazole (N_1), dibenzofuran (O_1), dibenzothiophene (S_1), carboline (N_2), perimidine (N_2), pyridoindole (N_2); and,

C_{14} (with 3 fused rings) derived from acridine (N_1), xanthene (O_1), thioxanthene (S_1), oxanthrene (O_2), phenoxathiin (O_1S_1),

10 phenazine (N_2), phenoxazine (N_1O_1), phenothiazine (N_1S_1), thianthrene (S_2), phenanthridine (N_1), phenanthroline (N_2), phenazine (N_2).

The above groups, whether alone or part of another substituent,
15 may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.
20
Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkoxy group, discussed
25 below), a C_{3-20} heterocyclyl group (also referred to as a C_{3-20} heterocyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7}alkyl group.

Alkoxy: -OR, wherein R is an alkyl group, for example, a C_{1-7} alkyl
30 group. Examples of C_{1-7} alkoxy groups include, but are not limited to, -OMe (methoxy), -OEt (ethoxy), -O(nPr) (n-propoxy), -O(iPr) (isopropoxy), -O(nBu) (n-butoxy), -O(sBu) (sec-butoxy), -O(iBu) (isobutoxy), and -O(tBu) (tert-butoxy).

35 Acetal: $-CH(OR^1)(OR^2)$, wherein R^1 and R^2 are independently acetal substituents, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group, or, in

the case of a "cyclic" acetal group, R^1 and R^2, taken together
with the two oxygen atoms to which they are attached, and the
carbon atoms to which they are attached, form a heterocyclic ring
having from 4 to 8 ring atoms. Examples of acetal groups

5   include, but are not limited to, $-CH(OMe)_2$, $-CH(OEt)_2$, and
$-CH(OMe)(OEt)$.

Hemiacetal: $-CH(OH)(OR^1)$, wherein R^1 is a hemiacetal substituent,
for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a

10   C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of
hemiacetal groups include, but are not limited to, $-CH(OH)(OMe)$
and $-CH(OH)(OEt)$.

Ketal: $-CR(OR^1)(OR^2)$, where R^1 and R^2 are as defined for acetals,

15   and R is a ketal substituent other than hydrogen, for example, a
C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group,
preferably a C_{1-7} alkyl group. Examples ketal groups include, but
are not limited to, $-C(Me)(OMe)_2$, $-C(Me)(OEt)_2$, $-C(Me)(OMe)(OEt)$,
$-C(Et)(OMe)_2$, $-C(Et)(OEt)_2$, and $-C(Et)(OMe)(OEt)$.

20

Hemiketal: $-CR(OH)(OR^1)$, where R^1 is as defined for hemiacetals,
and R is a hemiketal substituent other than hydrogen, for
example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20}
aryl group, preferably a C_{1-7} alkyl group. Examples of hemiacetal

25   groups include, but are not limited to, $-C(Me)(OH)(OMe)$, $-$
$C(Et)(OH)(OMe)$, $-C(Me)(OH)(OEt)$, and $-C(Et)(OH)(OEt)$.

Oxo (keto, -one): $=O$.

30   Thione (thioketone): $=S$.

Imino (imine): $=NR$, wherein R is an imino substituent, for
example, hydrogen, C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or
a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group.

35   Examples of ester groups include, but are not limited to, $=NH$,
$=NMe$, $=NEt$, and $=NPh$.

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Formyl (carbaldehyde, carboxaldehyde): $-C(=O)H$.

Acyl (keto): $-C(=O)R$, wherein R is an acyl substituent, for example, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylacyl or
5   C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, $-C(=O)CH_3$ (acetyl), $-C(=O)CH_2CH_3$ (propionyl), $-C(=O)C(CH_3)_3$ (t-butyryl), and $-C(=O)Ph$ (benzoyl,
10   phenone).

Carboxy (carboxylic acid): $-C(=O)OH$.

Thiocarboxy (thiocarboxylic acid): $-C(=S)SH$.
15

Thiolocarboxy (thiolocarboxylic acid): $-C(=O)SH$.

Thionocarboxy (thionocarboxylic acid): $-C(=S)OH$.

20   Imidic acid: $-C(=NH)OH$.

Hydroxamic acid: $-C(=NOH)OH$.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl):
25     $-C(=O)OR$, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and $-C(=O)OPh$.
30

Acyloxy (reverse ester): $-OC(=O)R$, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of acyloxy groups include, but are not limited to,
35     $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, $-OC(=O)Ph$, and $-OC(=O)CH_2Ph$.

Oxycarboyloxy: $-OC(=O)OR$, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, $-OC(=O)OCH_3$,

5 $-OC(=O)OCH_2CH_3$, $-OC(=O)OC(CH_3)_3$, and $-OC(=O)OPh$.

Amino: $-NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20}

10   heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case of a "cyclic" amino group, R^1 and R^2, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Amino groups may be primary ($-NH_2$), secondary ($-NHR^1$), or tertiary ($-$

15   NHR^1R^2), and in cationic form, may be quaternary ($-^+NR^1R^2R^3$). Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and $-NHPh$. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and

20   thiomorpholino.

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido

25   groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl,

30   thiomorpholinocarbonyl, and piperazinocarbonyl.

Thioamido (thiocarbamyl): $-C(=S)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to,

35   $-C(=S)NH_2$, $-C(=S)NHCH_3$, $-C(=S)N(CH_3)_2$, and $-C(=S)NHCH_2CH_3$.

14

Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group, and R^2 is an acyl substituent, for example, a C_{1-7}

5   alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and $-NHC(=O)Ph$. R^1 and R^2 may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl,

10   and phthalimidyl:

succinimidyl   maleimidyl   phthalimidyl
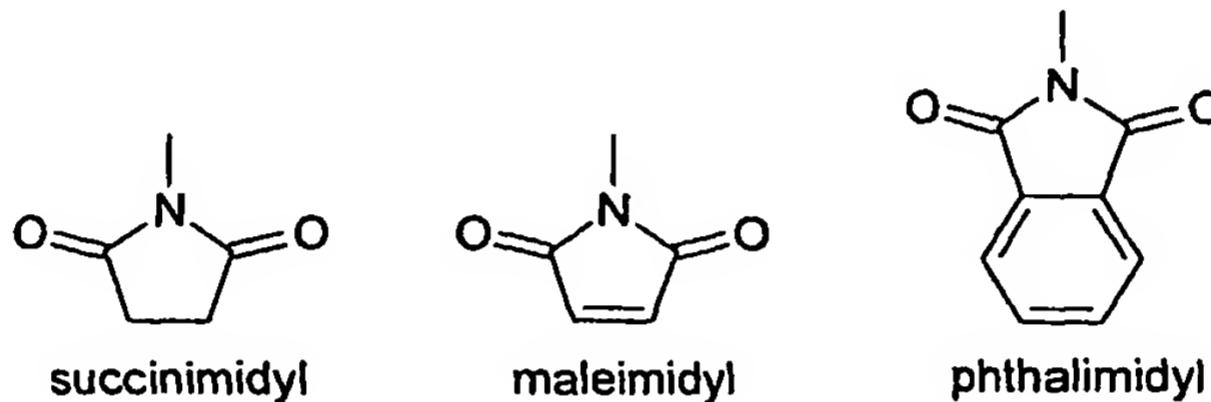
Aminocarbonyloxy: $-OC(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of

15   aminocarbonyloxy groups include, but are not limited to, $-OC(=O)NH_2$, $-OC(=O)NHMe$, $-OC(=O)NMe_2$, and $-OC(=O)NEt_2$.

Ureido: $-N(R^1)CONR^2R^3$ wherein R^2 and R^3 are independently amino substituents, as defined for amino groups, and R^1 is a ureido

20   substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of ureido groups include, but are not limited to, $-NHCONH_2$, $-NHCONHMe$, $-NHCONHEt$, $-NHCONMe_2$, $-NHCONEt_2$, $-NMeCONH_2$, $-NMeCONHMe$, $-NMeCONHEt$, $-NMeCONMe_2$, and $-NMeCONEt_2$.

25

Guanidino: $-NH-C(=NH)NH_2$.

Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom,
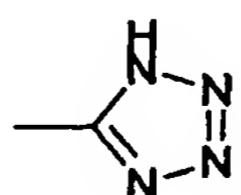
30

Imino: $\cdot$ =NR, wherein R is an imino substituent, for example, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group. Examples of imino groups include, but are not limited to, =NH, =NMe, and

5   =NEt.

Amidine (amidino): $-C(=NR)NR_2$, wherein each R is an amidine substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7}

10   alkyl group. Examples of amidine groups include, but are not limited to, $-C(=NH)NH_2$, $-C(=NH)NMe_2$, and $-C(=NMe)NMe_2$.

Nitro: $-NO_2$.

15   Nitroso: $-NO$.

Azido: $-N_3$.

Cyano (nitrile, carbonitrile): $-CN$.

20

Isocyano: $-NC$.

Cyanato: $-OCN$.

25   Isocyanato: $-NCO$.

Thiocyano (thiocyanato): $-SCN$.

Isothiocyano (isothiocyanato): $-NCS$.

30

Sulfhydryl (thiol, mercapto): $-SH$.

Thioether (sulfide): $-SR$, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio

35   group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Disulfide: $-SS-R$, wherein R is a disulfide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group (also referred to herein

5   as C_{1-7} alkyl disulfide). Examples of C_{1-7} alkyl disulfide groups include, but are not limited to, $-SSCH_3$ and $-SSCH_2CH_3$.

Sulfine (sulfinyl, sulfoxide): $-S(=O)R$, wherein R is a sulfine substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl

10   group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfine groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent,

15   for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group, including, for example, a fluorinated or perfluorinated C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$ (triflyl),

20   $-S(=O)_2CH_2CH_3$ (esyl), $-S(=O)_2C_4F_9$ (nonaflyl), $-S(=O)_2CH_2CF_3$ (tresyl), $-S(=O)_2CH_2CH_2NH_2$ (tauryl), $-S(=O)_2Ph$ (phenylsulfonyl, besyl), 4-methylphenylsulfonyl (tosyl), 4-chlorophenylsulfonyl (closyl), 4-bromophenylsulfonyl (brosyl), 4-nitrophenyl (nosyl), 2-naphthalenesulfonate (napsyl), and 5-dimethylamino-naphthalen-

25   1-ylsulfonate (dansyl).

Sulfinic acid (sulfino): $-S(=O)OH$, $-SO_2H$.

Sulfonic acid (sulfo): $-S(=O)_2OH$, $-SO_3H$.

30

Sulfinate (sulfinic acid ester): $-S(=O)OR$; wherein R is a sulfinate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinate groups include, but are not limited

35   to, $-S(=O)OCH_3$ (methoxysulfinyl; methyl sulfinate) and $-S(=O)OCH_2CH_3$ (ethoxysulfinyl; ethyl sulfinate).

Sulfonate (sulfonic acid ester): $-S(=O)_2OR$, wherein R is a sulfonate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonate groups include, but are not limited

5 to, $-S(=O)_2OCH_3$ (methoxysulfonyl; methyl sulfonate) and $-S(=O)_2OCH_2CH_3$ (ethoxysulfonyl; ethyl sulfonate).

Sulfinyloxy: $-OS(=O)R$, wherein R is a sulfinyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a

10 C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinyloxy groups include, but are not limited to, $-OS(=O)CH_3$ and $-OS(=O)CH_2CH_3$.

Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent,

15 for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ (mesylate) and $-OS(=O)_2CH_2CH_3$ (esylate).

20 Sulfate: $-OS(=O)_2OR$; wherein R is a sulfate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfate groups include, but are not limited to, $-OS(=O)_2OCH_3$ and $-SO(=O)_2OCH_2CH_3$.

25

Sulfamyl (sulfamoyl; sulfinic acid amide; sulfinamide): $-S(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, $-S(=O)NH_2$, $-S(=O)NH(CH_3)$,

30 $-S(=O)N(CH_3)_2$, $-S(=O)NH(CH_2CH_3)$, $-S(=O)N(CH_2CH_3)_2$, and $-S(=O)NHPh$.

Sulfonamido (sulfinamoyl; sulfonic acid amide; sulfonamide): $-S(=O)_2NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of

35 sulfonamido groups include, but are not limited to, $-S(=O)_2NH_2$, $-S(=O)_2NH(CH_3)$, $-S(=O)_2N(CH_3)_2$, $-S(=O)_2NH(CH_2CH_3)$, $-S(=O)_2N(CH_2CH_3)_2$, and $-S(=O)_2NHPh$.

18

Sulfamino: $-NR^1S(=O)_2OH$, wherein R^1 is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, $-NHS(=O)_2OH$ and $-N(CH_3)S(=O)_2OH$.

Sulfonamino: $-NR^1S(=O)_2R$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonamino groups include, but are not limited to, $-NHS(=O)_2CH_3$ and $-N(CH_3)S(=O)_2C_6H_5$.

Sulfinamino: $-NR^1S(=O)R$, wherein R^1 is an amino substituent, as defined for amino groups, and R is a sulfinamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinamino groups include, but are not limited to, $-NHS(=O)CH_3$ and $-N(CH_3)S(=O)C_6H_5$.

Phosphino (phosphine): $-PR_2$, wherein R is a phosphino substituent, for example, $-H$, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably $-H$, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphino groups include, but are not limited to, $-PH_2$, $-P(CH_3)_2$, $-P(CH_2CH_3)_2$, $-P(t-Bu)_2$, and $-P(Ph)_2$.

Phospho: $-P(=O)_2$.

Phosphinyl (phosphine oxide): $-P(=O)R_2$, wherein R is a phosphinyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group or a C_{5-20} aryl group. Examples of phosphinyl groups include, but are not limited to, $-P(=O)(CH_3)_2$, $-P(=O)(CH_2CH_3)_2$, $-P(=O)(t-Bu)_2$, and $-P(=O)(Ph)_2$.

Phosphonic acid (phosphono): $-P(=O)(OH)_2$.

Phosphonate (phosphono ester): $-P(=O)(OR)_2$, where R is a phosphonate substituent, for example, $-H$, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably $-H$, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphonate groups

5   include, but are not limited to, $-P(=O)(OCH_3)_2$, $-P(=O)(OCH_2CH_3)_2$, $-P(=O)(O-t-Bu)_2$, and $-P(=O)(OPh)_2$.

Phosphoric acid (phosphonooxy): $-OP(=O)(OH)_2$.

10 Phosphate (phosphonooxy ester): $-OP(=O)(OR)_2$, where R is a phosphate substituent, for example, $-H$, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably $-H$, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphate groups include, but are not limited to, $-OP(=O)(OCH_3)_2$, $-OP(=O)(OCH_2CH_3)_2$,

15 $-OP(=O)(O-t-Bu)_2$, and $-OP(=O)(OPh)_2$.

Phosphorous acid: $-OP(OH)_2$.

Phosphite: $-OP(OR)_2$, where R is a phosphite substituent, for

20 example, $-H$, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably $-H$, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphite groups include, but are not limited to, $-OP(OCH_3)_2$, $-OP(OCH_2CH_3)_2$, $-OP(O-t-Bu)_2$, and $-OP(OPh)_2$.

25 Phosphoramidite: $-OP(OR^1)-NR^2_2$, where R^1 and R^2 are phosphoramidite substituents, for example, $-H$, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably $-H$, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidite groups include, but are not limited to,

30 $-OP(OCH_2CH_3)-N(CH_3)_2$, $-OP(OCH_2CH_3)-N(i-Pr)_2$, and $-OP(OCH_2CH_2CN)-N(i-Pr)_2$.

Phosphoramidate: $-OP(=O)(OR^1)-NR^2_2$, where R^1 and R^2 are phosphoramidate substituents, for example, $-H$, a (optionally

35 substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably $-H$, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidate groups include, but are not

limited to, $-OP(=O)(OCH_2CH_3)-N(CH_3)_2$, $-OP(=O)(OCH_2CH_3)-N(i-Pr)_2$, and $-OP(=O)(OCH_2CH_2CN)-N(i-Pr)_2$.

Alkylene

5   C_{3-12} alkylene: The term "C_{3-12} alkylene", as used herein, pertains
    to a bidentate moiety obtained by removing two hydrogen atoms,
    either both from the same carbon atom, or one from each of two
    different carbon atoms, of a hydrocarbon compound having from 3
    to 12 carbon atoms (unless otherwise specified), which may be
10 aliphatic or alicyclic, and which may be saturated, partially
    unsaturated, or fully unsaturated.   Thus, the term "alkylene"
    includes the sub-classes alkenylene, alkynylene, cycloalkylene,
    etc., discussed below.

15 Examples of linear saturated C_{3-12} alkylene groups include, but are
    not limited to, $-(CH_2)_n-$ where n is an integer from 3 to 12, for
    example, $-CH_2CH_2CH_2-$ (propylene), $-CH_2CH_2CH_2CH_2-$ (butylene),
    $-CH_2CH_2CH_2CH_2CH_2-$ (pentylene) and $-CH_2CH_2CH_2CH_2CH_2CH_2CH_2-$ (heptylene).

20 Examples of branched saturated C_{3-12} alkylene groups include, but
    are not limited to, $-CH(CH_3)CH_2-$, $-CH(CH_3)CH_2CH_2-$,
    $-CH(CH_3)CH_2CH_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-CH_2CH(CH_3)CH_2CH_2-$, $-CH(CH_2CH_3)-$,
    $-CH(CH_2CH_3)CH_2-$, and $-CH_2CH(CH_2CH_3)CH_2-$.

25 Examples of linear partially unsaturated C_{3-12} alkylene groups (C_{3-12} alkenylene, and alkynylene groups) include, but are not limited
    to, $-CH=CH-CH_2-$, $-CH_2-CH=CH_2-$, $-CH=CH-CH_2-CH_2-$, $-CH=CH-CH_2-CH_2-CH_2-$,
    $-CH=CH-CH=CH-$, $-CH=CH-CH=CH-CH_2-$, $-CH=CH-CH=CH-CH_2-CH_2-$, $-CH=CH-$
    $CH_2-CH=CH-$, $-CH=CH-CH_2-CH_2-CH=CH-$, and $-CH_2-C{\equiv}C-CH_2-$.

30
    Examples of branched partially unsaturated C_{3-12} alkylene groups
    (C_{3-12} alkenylene and alkynylene groups) include, but are not
    limited to, $-C(CH_3)=CH-$, $-C(CH_3)=CH-CH_2-$, $-CH=CH-CH(CH_3)-$ and $-C{\equiv}C-$
    $CH(CH_3)-$.

35
    Examples of alicyclic saturated C_{3-12} alkylene groups (C_{3-12}
    cycloalkylenes) include, but are not limited to, cyclopentylene

(e.g. cyclopent-1,3-ylene), and cyclohexylene
(e.g. cyclohex-1,4-ylene).

Examples of alicyclic partially unsaturated C_{3-12} alkylene groups
(5) C_{3-12} cycloalkylenes) include, but are not limited to,
cyclopentenylene (e.g. 4-cyclopenten-1,3-ylene), cyclohexenylene
(e.g. 2-cyclohexen-1,4-ylene; 3-cyclohexen-1,2-ylene;
2,5-cyclohexadien-1,4-ylene).

10 Includes Other Forms
Unless otherwise specified, included in the above are the well
known ionic, salt, solvate, and protected forms of these
substituents. For example, a reference to carboxylic acid
(-COOH) also includes the anionic (carboxylate) form (-COO⁻), a
15 salt or solvate thereof, as well as conventional protected forms.
Similarly, a reference to an amino group includes the protonated
form (-N⁺HR¹R²), a salt or solvate of the amino group, for
example, a hydrochloride salt, as well as conventional protected
forms of an amino group. Similarly, a reference to a hydroxyl
20 group also includes the anionic form (-O⁻), a salt or solvate
thereof, as well as conventional protected forms.

Isomers, Salts, Solvates, Protected Forms, and Prodrugs
Certain compounds may exist in one or more particular geometric,
25 optical, enantiomeric, diasteriomeric, epimeric, atropic,
stereoisomeric, tautomeric, conformational, or anomeric forms,
including but not limited to, cis- and trans-forms; E- and Z-
forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and
meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms;
30 keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal-
and anticlinal-forms; α- and β-forms; axial and equatorial forms;
boat-, chair-, twist-, envelope-, and halfchair-forms; and
combinations thereof, hereinafter collectively referred to as
"isomers" (or "isomeric forms").

35
Note that, except as discussed below for tautomeric forms,
specifically excluded from the term "isomers", as used herein,

are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, -OCH$_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH$_2$OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C$_{1-7}$ alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.

keto   enol   enolate
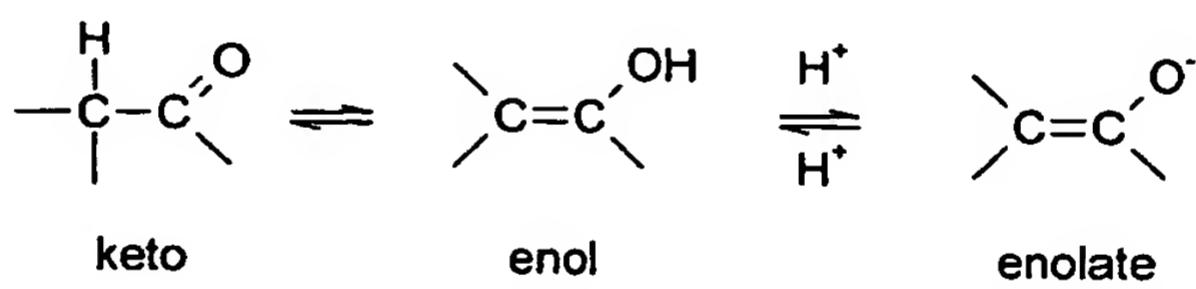
Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

23

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

5 It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977).

10

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be -COO$^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal

15 ions such as Na$^+$ and K$^+$, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{+3}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. NH$_4$$^+$) and substituted ammonium ions (e.g. NH$_3$R$^+$, NH$_2$R$_2$$^+$, NHR$_3$$^+$, NR$_4$$^+$). Examples of some suitable substituted ammonium ions are those

20 derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary

25 ammonium ion is N(CH$_3$)$_4$$^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g. -NH$_2$ may be -NH$_3$$^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions

30 include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

Examples of suitable organic anions include, but are not limited

35 to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic,

24

ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic,
glycolic, hydroxymaleic, hydroxynaphthalene carboxylic,
isethionic, lactic, lactobionic, lauric, maleic, malic,
methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic,
5 pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic,
salicylic, stearic, succinic, sulfanilic, tartaric,
toluenesulfonic, and valeric. Examples of suitable polymeric
organic anions include, but are not limited to, those derived
from the following polymeric acids: tannic acid, carboxymethyl
10 cellulose.

It may be convenient or desirable to prepare, purify, and/or
handle a corresponding solvate of the active compound. The term
"solvate" is used herein in the conventional sense to refer to a
15 complex of solute (e.g. active compound, salt of active compound)
and solvent. If the solvent is water, the solvate may be
conveniently referred to as a hydrate, for example, a mono-
hydrate, a di-hydrate, a tri-hydrate, etc.

20 Solvates of particular relevance to the present invention are
those where the solvent adds across the imine bond of the PBD
moiety, which is illustrated below where the solvent is water or
an alcohol (R^{A}OH, where R^A is an ether substituent as described
above):
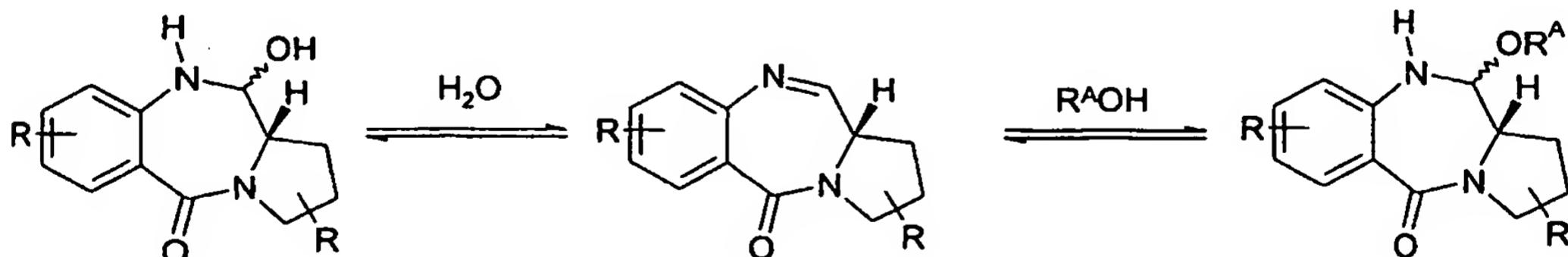
25

These forms can be called the carbinolamine and carbinolamine
ether forms of the PBD. The balance of these equilibria depend
on the conditions in which the compounds are found, as well as
the nature of the moiety itself.

30

In general any nuceleophilic solvent is capable of forming such
solvates as illustrated above for hydoxylic solvents. Other
nuceleophilic solvents include thiols and amines.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form" is used herein in the

5   conventional chemical sense and pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions under specified conditions (e.g. pH, temperature, radiation, solvent, and the like). In practice, well known chemical methods are employed to reversibly render

10 unreactive a functional group, which otherwise would be reactive, under specified conditions. In a chemically protected form, one or more reactive functional groups are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional

15 group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, "Protective Groups in Organic

20 Synthesis" (Green, T. and Wuts, P.; 3rd Edition; John Wiley and Sons, 1999).

A wide variety of such "protecting", "blocking", or "masking" methods are widely used and well known in organic synthesis. For

25 example, a compound which has two nonequivalent reactive functional groups, both of which would be reactive under specified conditions, may be derivatized to render one of the functional groups "protected", and therefore unreactive, under the specified conditions; so protected, the compound may be used

30 as a reactant which has effectively only one reactive functional group. After the desired reaction (involving the other functional group) is complete, the protected group may be "deprotected" to return it to its original functionality.

35 For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl)

ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH₃, -OAc).

For example, an aldehyde or ketone group may be protected as an
5 acetal (R-CH(OR)₂) or ketal (R₂C(OR)₂), respectively, in which the carbonyl group (>C=O) is converted to a diether (>C(OR)₂), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.
10

For example, an amine group may be protected, for example, as an amide (-NRCO-R) or a urethane (-NRCO-OR), for example, as: a methyl amide (-NHCO-CH₃); a benzyloxy amide (-NHCO-OCH₂C₆H₅, -NH-Cbz); as a t-butoxy amide (-NHCO-OC(CH₃)₃, -NH-Boc); a 2-biphenyl-
15 2-propoxy amide (-NHCO-OC(CH₃)₂C₆H₄C₆H₅, -NH-Bpoc), as a 9-fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec);
20 or, in suitable cases (e.g., cyclic amines), as a nitroxide radical (>N-O·).

For example, a carboxylic acid group may be protected as an ester for example, as: an C₁₋₇ alkyl ester (e.g. a methyl ester; a t-
25 butyl ester); a C₁₋₇ haloalkyl ester (e.g. a C₁₋₇ trihaloalkyl ester); a triC₁₋₇ alkylsilyl-C₁₋₇ alkyl ester; or a C₅₋₂₀ aryl-C₁₋₇ alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

30 For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-S-CH₂NHC(=O)CH₃).

It may be convenient or desirable to prepare, purify, and/or
35 handle the active compound in the form of a prodrug. The term "prodrug", as used herein, pertains to a compound which, when metabolised (e.g. *in vivo*), yields the desired active compound.

Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

5   For example, some prodrugs are esters of the active compound (e.g. a physiologically acceptable metabolically labile ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in
10   the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required.

Also, some prodrugs are activated enzymatically to yield the
15   active compound, or a compound which, upon further chemical reaction, yields the active compound (for example, as in ADEPT, GDEPT, LIDEPT, etc.). For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.
20

In particular, prodrugs of pyrrolobenzodiazepines are described in WO 00/12507, which is incorporated herein by reference. In these prodrugs a nitrogen protecting group which can be removed *in vivo* (e.g. enzymatically, using light) is bound to the
25   nitrogen of the imine group, with the carbon of the imine group bearing a hydroxy, ester or thioester group. Examples of this form of protection include:
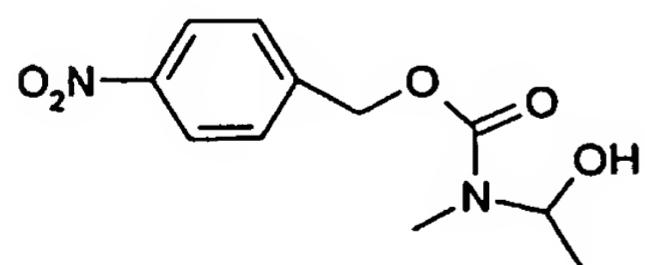
, which is nitroreductase labile (e.g. using
30   ADEPT/GDEPT);

and , which are photolabile;
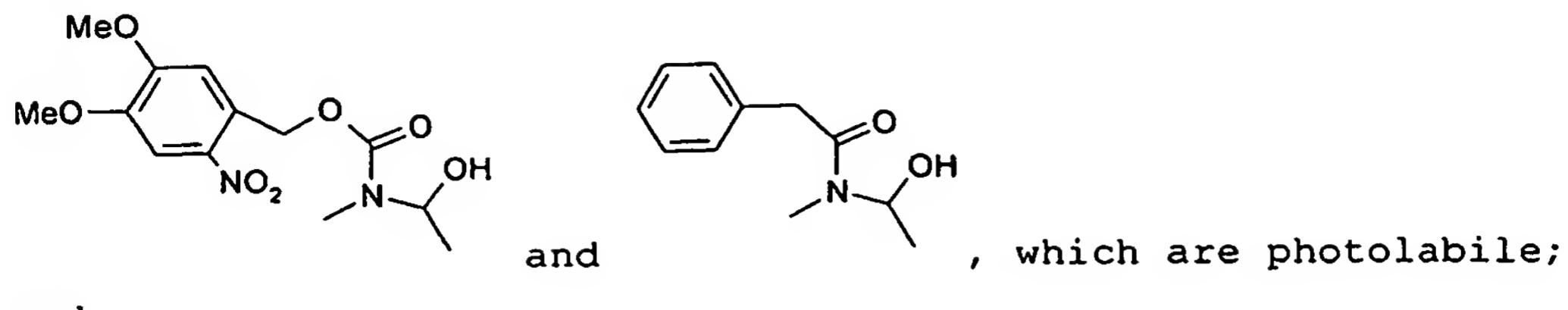
and
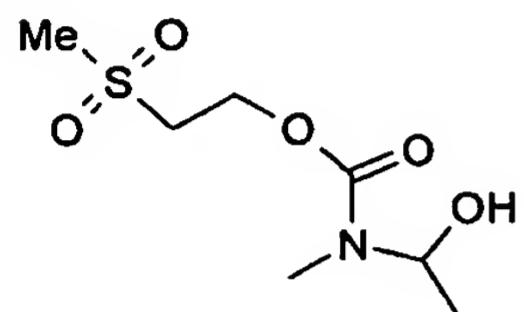
which is glutathione labile (e.g. using NPEPT).

5

General synthetic routes

A key intermediate in the synthesis of the compounds of the present invention is the following compound, an enol triflate, of formula (A):

(A)
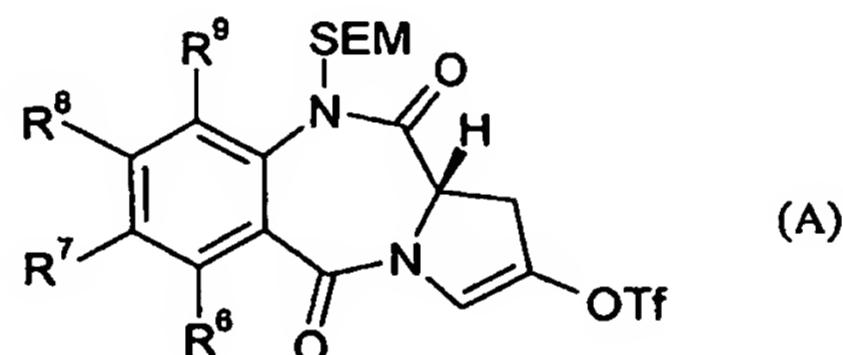
10

wherein the groups R^6 to R^9 are as for the final product, SEM represents a trimethyl silyl ethoxy methyl group, and OTf represents the group OSO_2CF_3. The synthesis of this compound is described in detail in WO 00/12508, which is incorporated herein

15 by reference. In particular, reference is made to scheme 7 on page 24, where the above compound is designated as intermediate **P**. The SEM protecting group can be replaced by other suitable nitrogen protecting groups.

20 The route from this compound to the desired final product is illustrated in scheme 1.

29
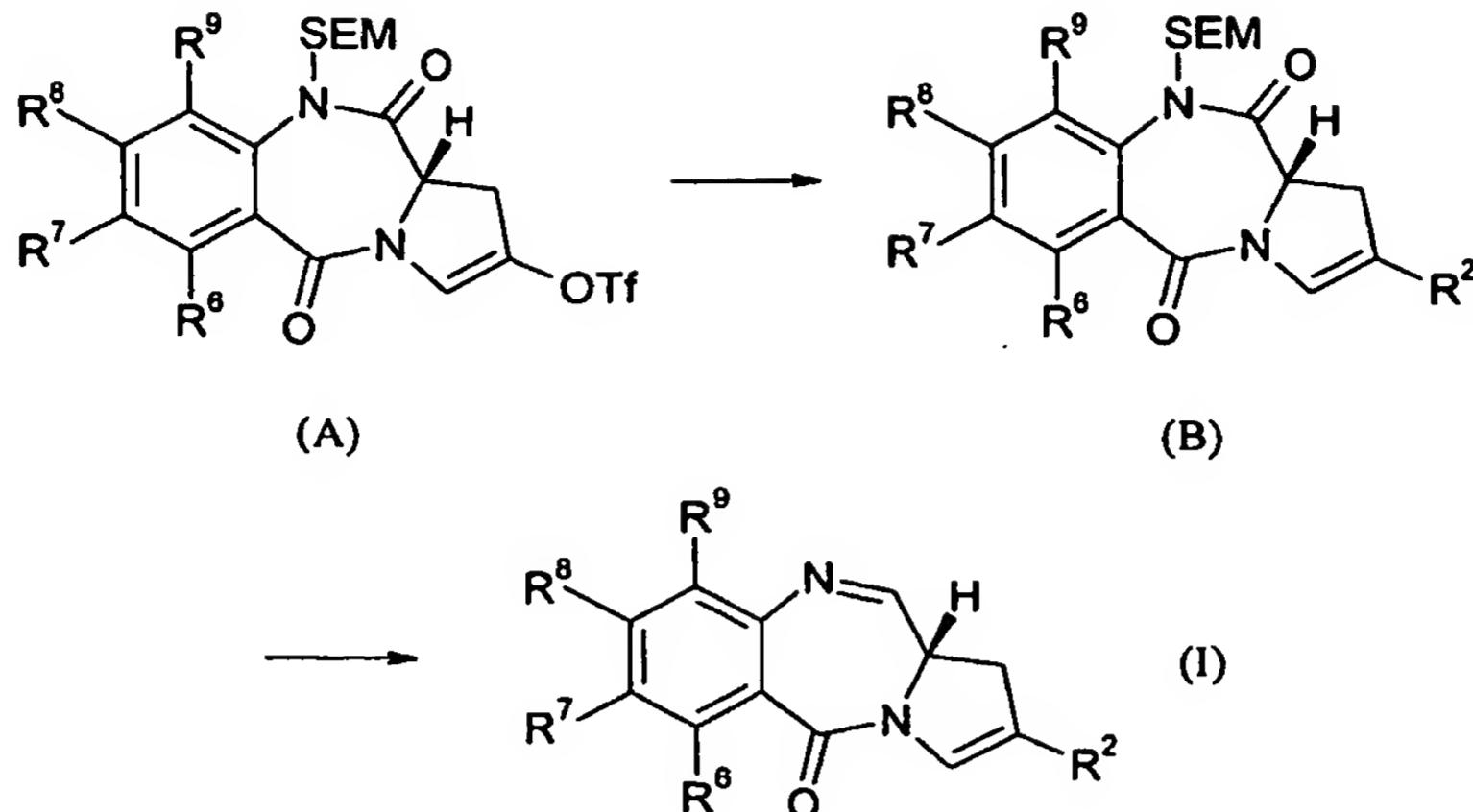
Scheme 1

The key enol triflate intermediate (**A**) can be converted to the

5   appropriate SEM protected dilactam (**B**) by use of a Suzuki
coupling reaction, i.e. palladium catalysed cross coupling of the
enol triflate (**A**) with the appropriate arylboron derivative.

The SEM protected dilactam (**B**) can then·by converted to the

10   desired final compound of formula (**I**) by reduction, which leads
to a protected carbinolamine intermediate, which can then be
treated to remove the SEM protecting group. The reduction of the
dilactam (**B**) can be accomplished by, for example, sodium
tetraborohydride, whilst a suitable means for removing the SEM

15   protecting group is treatment with silica gel.

This synthesis route is equally applicable to the synthesis of
dimers.

20   Further Uses
The present invention also provides compounds as described in the
first aspect of the invention which regulate (e.g. inhibit) cell
proliferation. Thus, the present invention also provides methods
of regulating (e.g. inhibiting) cell proliferation, *in vitro* or

25   *in vivo,* comprising contacting a cell with an effective amount of
a compound of the first aspect of the invention.

In particular, this inhibition may be of certain cell lines as disclosed in the examples.

5 One of ordinary skill in the art is readily able to determine whether or not a candidate compound regulate (e.g. inhibit) cell proliferation. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described in the examples below.

10 For example, a sample of cells (e.g. from a tumour) may be grown *in vitro* and a test compound brought into contact with said cells, and the effect of the compound on those cells observed. As an example of "effect" the morphological status of the cells (e.g. alive or dead, etc.) may be determined. Where the test

15 compound is found to exert an influence on the cells, this may be used as a prognostic or diagnostic marker of the efficacy of the compound in methods of treating a patient carrying cells of the same cellular type.

20 Methods of Treatment

As described above, the second aspect of the invention provide the use of a compound of the first aspect of the invention in a method of therapy. Also provided is a method of treatment, comprising administering to a subject in need of treatment a

25 therapeutically-effective amount of a compound of the first aspect of the invention, preferably in the form of a pharmaceutical composition, which is the third aspect of the present invention. The term "therapeutically effective amount" is an amount sufficient to show benefit to a patient. Such

30 benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and

35 other medical doctors.

A compound may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Examples of treatments and therapies include, but are not limited to, chemotherapy (the

5   administration of active agents, including, e.g. drugs; surgery; and radiation therapy.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may

10   comprise, in addition to the active ingredient, i.e. a compound of formula I, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The

15   precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

Pharmaceutical compositions for oral administration may be in

20   tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide

25   solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such a gelatin.

For intravenous, cutaneous or subcutaneous injection, or

30   injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles

35   such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers,

antioxidants and/or other additives may be included, as required.

Preferences

The following preferences may apply to all aspects of the
5   invention as described above, or may relate to a single aspect.
The preferences may be combined together in any combination.

R^6 to R^9

If the compound is a dimer, it is preferred that the dimer bridge
10   is of formula $-O-(CH_2)_n-O-$, where n is from 3 to 12, and more
preferably 3 to 7.

R^9 is preferably H.

15   R^6 is preferably selected from H, OH, OR, SH, NH_2, nitro and halo,
and is more preferably H or halo, and most preferably is H.

R^7 and R^8 (when the compound is not a dimer) are preferably
independently selected from H, OH, OR, SH, SR, NH_2, NHR, NHRR',
20   and halo, and more preferably independently selected from H, OH
and OR, where R is preferably selected from optionally
substituted C_{1-7} alkyl, C_{3-10} heterocyclyl and C_{5-10} aryl groups.
Particularly preferred substituents at the 7- and 8- positions
are OMe and OCH_2Ph.

25

R^2

In one set of embodiments R^2 is an optionally substituted napthyl
group. Preferred substituents include: halo, more particularly
chloro and fluoro; C_{1-7} alkyl groups, more particularly C_{1-4} alkyl
30   groups, e.g. methyl and t-butyl; ether groups, more particularly
C_{1-4} alkoxy groups, e.g. methoxy and benzyloxy; C_{5-20} aryl groups,
particularly phenyl.

The napthyl group may be linked to the main PBD moiety at any
35   position on its ring system. For example, if the napthyl is
unsubstituted, then the group may either be napth-1-yl or napth-

2-yl, with napth-2-yl being preferred.

Especially preferred napthyl groups include: napth-2-yl, 6-methoxy-napth-2-yl, 6-ethoxy-napth-2-yl and fluoro and/or chloro
5   substituted napthyl groups, with single substituted fluoro or chloro napth-2-yls being most preferred.

The optional substituents may also be at any ring position. It is preferred that there is not one substituent group either side
10 of the bond to the main PBD moiety.

In one set of embodiments R^2 is an optionally substituted thiophenyl group. Preferred substituents include: halo, more particularly chloro and fluoro; C_{1-7} alkyl groups, more
15 particularly C_{1-4} alkyl groups, e.g. methyl and t-butyl; ether groups, more particularly C_{1-4} alkoxy groups, e.g. methoxy and benzyloxy; C_{5-20} aryl groups, particularly phenyl.

The thiophenyl group may be linked to the main PBD moiety at any
20 available position on its ring system. For example, if the thiophenyl is unsubstituted, then the group may either be thiophen-2-yl or thiophen-3-yl, with thiophen-2-yl being preferred.

25 Especially preferred thiophenyl groups include: thiophen-2-yl, 5-chloro-thiophen-2-yl and 5-methyl-thiophen-2-yl.

The optional substituents may also be at any ring position. If the thiophenyl group is thiophen-3-yl, then it is preferred that
30 there is not one substituent group either side of the bond to the main PBD moiety.

In one set of embodiments R^2 is an optionally substituted furanyl group. Preferred substituents include: halo, more particularly
35 chloro and fluoro; C_{1-7} alkyl groups, more particularly C_{1-4} alkyl groups, e.g. methyl and t-butyl; ether groups, more particularly

C_{1-4} alkoxy groups, e.g. methoxy and benzyloxy; C_{5-20} aryl groups, particularly phenyl.

The furanyl group may be linked to the main PBD moiety at any available position on its ring system. For example, if the furanyl is unsubstituted, then the group may either be furan-2-yl or furan-3-yl, with furan-2-yl being preferred.

Especially preferred furanyl groups include: furan-2-yl, 5-chloro-furan-2-yl and 5-methyl-furan-2-yl.

The optional substituents may also be at any ring position. If the furanyl group is furan-3-yl, then it is preferred that there is not one substituent group either side of the bond to the main PBD moiety.

In another set of embodiments R^2 is a phenyl group substituted by an ethyl or propyl group, preferably an ethyl or n-propyl group and more preferably an ethyl group. These groups are preferably in the ortho (2-) or para (4-) positions, with ortho (2-) being more preferred.

Thus an especially preferred R^2 group of this type is 4-ethylphenyl.

In another set of embodiments R^2 is a phenyl group substituted by one or more chloro or fluoro groups. If there is only one substituent group this is preferably in the ortho (2-) or para (4-) positions, and more preferably in the para (4-) position. If there are two or more substituents, in general the ortho and para positions are the preferred substituent sites. However, it is preferred that there are not two chloro substituents at the 2- and 6- positions. It is also preferred that all the substituents are either fluoro or chloro.

Especially preferred R^2 groups of this type are: 4-chlorophenyl; 4-fluorophenyl; 2-chlorophenyl; 2-fluorophenyl; 2,6-

difluorophenyl; and 3,4-dichlorophenyl.

The following compounds are particularly preferred:
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-napthyl)-5H-pyrrolo[2,1-

5   c][1,4]benzodiazepin-5-one (**11**);
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-$tert$-butylbenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**13**);
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-chlorobenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**15**);

10 (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-fluorobenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**17**);
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-methylbenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**19**);
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-ethylbenzene)-5H-

15 pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**21**);
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-thiophenyl)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**23**);
(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-furan)-5H-pyrrolo[2,1-
c][1,4]benzodiazepin-5-one (**25**); and

20 (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2,6-dimethylbenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (**27**).

The compounds of the first aspect of the invention are preferably
used in treating, and in manufacturing medicaments to treat,

25 cancers, and in particular breast, renal and lung cancers and for
inhibiting the growth of breast, renal and lung cancer cell
lines.

In particular, compounds where R^2 is an optionally substitued

30 napthyl or thiophenyl group, are preferably used in treating, and
in manufacturing medicaments to treat, lung, breast and renal
cancer and melanomas, and more preferably breast cancer.

Compounds where R^2 is a phenyl group with an ethyl or propyl

35 substituent are preferably used in treating, and in manufacturing
medicaments to treat, lung, breast and renal cancer and

36

melanomas.

Compounds where R^2 is a phenyl group with one or more fluoro
substituents (preferably a 4-fluoro substituent) are preferably
5 used in treating, and in manufacturing medicaments to treat,
renal cancer.

Compounds where R^2 is a phenyl group with a 4-t-butyl substituent,
one or more chloro substituents (preferably a 4-chloro
10 substituent), or a 2-methyl substituent are preferably used in
treating, and in manufacturing medicaments to treat, breast
cancer.   Compounds where R^2 is a phenyl group with a 4-t-butyl
substituent are also preferably used in treating, and in
manufacturing medicaments to treat, melanomas and renal cancer.
15

Compounds where R^2 is a phenyl group with a 2-methyl substituent
are preferably used in treating, and in manufacturing medicaments
to treat, renal cancer.

20 Compounds where R^2 is a phenyl group with 2,6-dimethyl
substituents are preferably used in treating, and in
manufacturing medicaments to treat, renal cancer.

25 Examples
General Experimental Methods
Melting points (mp) were determined on a Gallenkamp P1384 digital
melting point apparatus and are uncorrected. Infrared (IR)
spectra were recorded using a Perkin-Elmer 297 spectrophotometer.
30 ^{1}H- and ^{13}C- NMR spectra were recorded on either a Jeol GSX 270
MHZ FT-NMR spectrometer or a Brucker AMX-250 MHz NMR spectrometer
operating at 20°C +/-1°C. Chemical shifts are reported in parts
per million (δ) downfield from tetramethylsilane (TMS). Spin
multiplicities are described as: s (singlet), bs (broad singlet),
35 d (doublet), dd (doublet of doublets), t (triplet), q (quartet),
p (pentuplet) or m (multiplet). Mass spectra (MS) were recorded
using a Jeol JMS-DX 303 GC Mass Spectrometer (EI mode: 70eV,

source 117-147°C). Accurate molecular masses (HRMS) were
determined by peak matching using perfluorokerosene (PFK) as an
internal mass marker, and FAB mass spectra were obtained from a
glycerol/thioglycerol/trifluoroacetic acid (1:1:0.1) matrix with
5   a source temperature of 180°C. Optical rotations at the Na-D
line were obtained at ambient temperature using a Perkin-Elmer
141 Polarimeter. Analytical results were generally within +/-
0.2% of the theoretical values. Flash chromatography was
performed using Aldrich flash chromatography Silica Gel-60 (E.
10   Merck, 230-400 mesh). Automated flash chromatography was carried
out on a Jones Flashmaster II. Thin-layer chromatography (TLC)
was performed using GF_{254} silica gel (with fluorescent indicator)
on glass plates. All solvents and reagents, unless otherwise
stated, were supplied by the Aldrich Chemical Company Ltd. and
15   were used as supplied without further purification. Anhydrous
solvents were prepared by distillation under a dry nitrogen
atmosphere in the presence of an appropriate drying agent, and
were stored over 4Å molecular sieves or sodium wire. Petroleum
ether refers to the fraction boiling at 40-60°C.

20

38

Synthesis of key intermediate
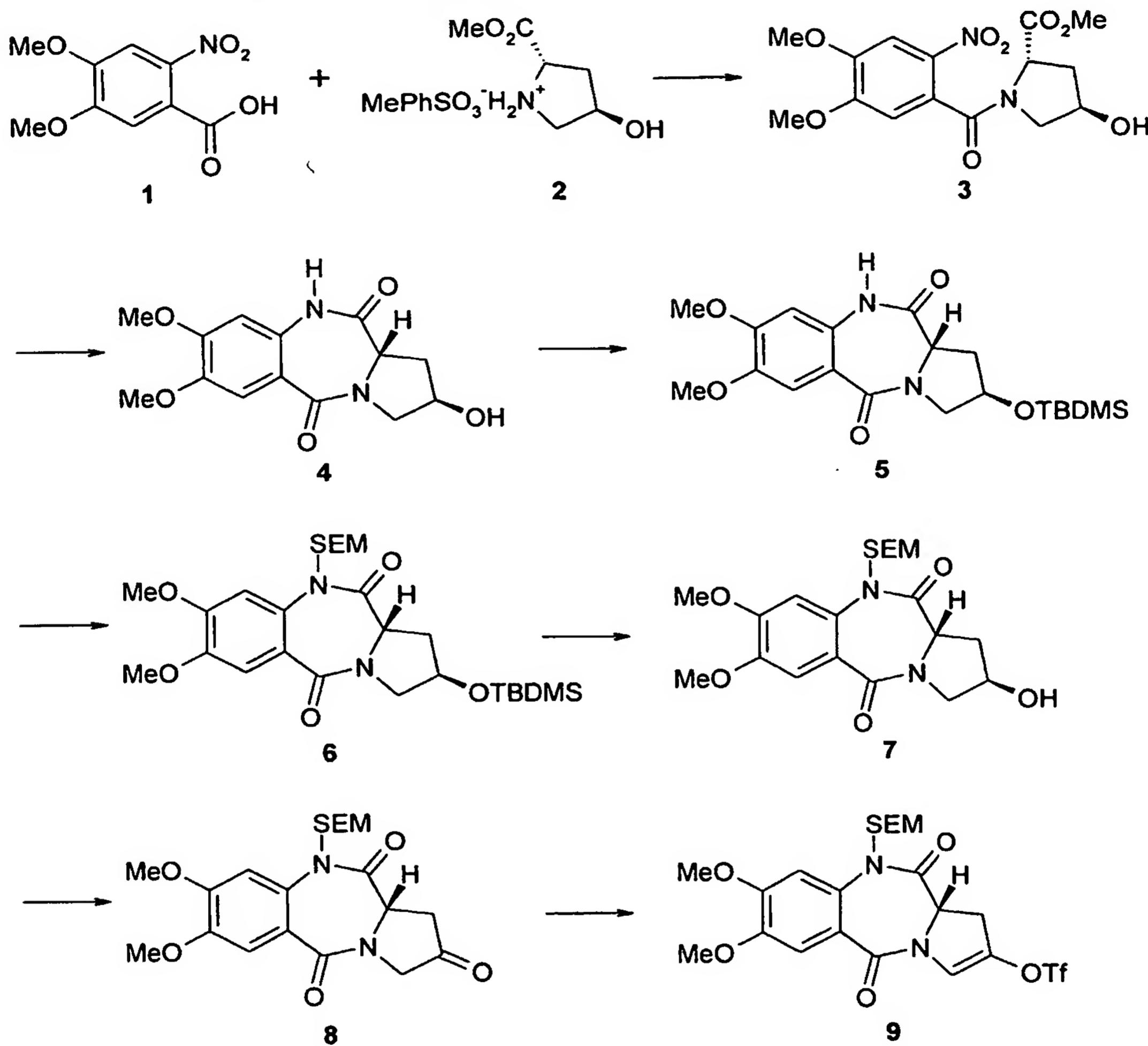
N-(4,5-dimethoxy-2-nitrobenzoyl)hydroxyproline methyl ester (3)

Oxalyl chloride (15.38 g, 121.11 mmol) was added in one portion
to a stirred suspension of 2-nitro-4,5-dimethoxybenzoic acid (1)
(25.01 g, 110.10 mmol) in anhydrous DCM (100 mL) at room

5 temperature. A catalytic amount of DMF (2 drops) was added and
the reaction mixture was allowed to stir for 16 hours under an
inert atmosphere. The acid chloride solution was added dropwise
to a vigorously stirred solution of the pyrrolo compound (2)
(34.90 g, 110.10 mmol; *JOC 5*, **13**, 3621 (1994)) and TEA (45.95 mL,

10 33.36 g, 330.29 mmol) in anhydrous DCM (100 mL) at -20°C. The
reaction mixture was allowed to stir for 16 hours at room
temperature. The reaction mixture was washed with saturated
$NaHCO_3$ (2 x 200 mL), saturated NH_4Cl (2 x 200 mL), water (2 x 200
mL), brine (2 x 200 mL) and dried over anhydrous $MgSO_4$.

15 Filtration and evaporation of the solvent *in vacuo* afforded the
crude product (3), which was purified by flash column
chromatography using EtOAc as eluent. Pure fractions were
combined and evaporation of excess eluent *in vacuo* afforded the
product as a foam (33.26 g, 93.9 mmol, 85%). ^1H NMR (270 MHz,

20 $CDCl_3$) δ 7.69 (s, 1H), 6.87 (s, 1H), 5.31 (s, 2H), 4.97-4.82 (m,
1H), 4.44 (br s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.81 (s, 3H),
3.54-3.48 (m, 1H), 3.18 (d, 1H, *J* = 2.02 Hz), 2.87 (br s, 1H),
2.45-2.16 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.6, 172.5,
167.5, 166.8, 154.4, 154.0, 149.3, 137.5, 137.4, 127.0, 126.2,

25 109.5, 107.2, 107.1, 69.9, 69.1, 59.2, 57.4, 56.9, 56.8, 56.6,
56.4, 54.6, 53.5, 52.5, 52.4, 39.4, 38.0.

(11aS)-6,7-dimethoxy-2(R)-hydroxy-2,3,5,10,11,11a-hexahydro-5,11-
dioxo-1H-pyrrolo[2,1-c][1,4-]benzodiazepine (4)

30 10% Pd/C catalyst (3.3 g) was added to a solution of **3** (33.0 g,
93.1 mmol) in absolute EtOH (250 mL). The reaction mixture was
hydrogenated under pressure using a Parr hydrogenator at 55 psi H_2
for 18 hours. The reaction mixture was filtered through celite,
and the celite washed with hot MeOH, taking care not to allow the

35 filter cake to dry out. Removal of excess solvent afforded the
crude product (20.14 g). The crude product was allowed to stir

in 1 N HCl (200 mL) and CHCl$_3$ (200 mL) for 30 minutes. The
organic layer was washed with 1 N HCl (100 mL) and the aqueous
layers were combined and neutralised with saturated aqueous
NaHCO$_3$. On leaving the aqueous extract overnight, a fine white
5 precipitate formed (4) which was collected by filtration and
dried (7.81 g, 26.72 mmol, 29 %). ^{1}H NMR (270 MHz, CDCl$_3$) δ 10.06
(s, 1H, NH), 7.61 (s, 1H, ArH), 7.36 (s, 1H, ArH), 4.49-4.41 (m,
1H, 2), 4.22-4.17 (m, 1H, 11a), 3.88 (s, 6H), 3.82-3.55 (m, 2H,
3), 3.20 (br s, 1H, OH), 2.87-2.77 (m, 1H, 1), 2.10-2.05 (m, 1H,
10 1); ^{13}C NMR (CDCl$_3$)δ 170.2, 165.9, 152.0, 145.7, 130.7, 118.2,
111.9, 104.2, 68.1, 56.0, 55.6, 54.2, 34.6, 18.8.

(11aS)-6,7-dimethoxy-2(R)-[(tert-butyldimethylsilyl)oxy]-
2,3,5,10,11,11a-hexahydro-5,11-dioxo-1H-pyrrolo[2,1-c][1,4-
15 *]benzodiazepine (5)*

Solid TBDMS Chloride (8.22 g, 54.44 mmol) was added in one
portion to a solution of 5 (7.23 g, 24.74 mmol) and imidazole
(8.42 g, 123.72 mmol) in anhydrous DMF (75 mL) and allowed to
stir at room temperature for 16 hours. The reaction mixture was
20 poured into water (500 mL) and filtered to afford the crude
product (5), which was purified by recrystallisation from EtOH
(800 mL) as fine white needles (6.995 g, 17.21 mmol, 70 %). ^{1}H
NMR (270 MHz, CDCl$_3$) δ 10.06 (s, 1H, NH), 7.37 (s, 1H, ArH), 6.68
(s, 1H, ArH), 4.19-4.14 (m, 1H, 2), 4.06-4.01 (m, 1H, 11a), 3.90
25 (s, 3H, OMe), 3.88 (s, 3H, OMe), 3.69-3.63 (m, 2H, 3), 2.85-2.80
(m, 1H, 1), 2.05-2.01 (m, 1H, 1); ^{13}C NMR (67.8 MHz, CDCl$_3$) δ
170.4, 170.2, 165.9, 152.1, 145.8, 145.6, 131.1, 130.7, 118.1,
111.9, 104.3, 104.1, 69.2, 69.1, 56.0, 55.9, 55.7, 54.3, 54.0,
35.0, 25.8, 25.7, 25.6, 17.9, -3.0, -3.5, -4.9, -5.0.

30

(11aS)-6,7-dimethoxy-2(R)-[(tert-butyldimethylsilyl)oxy]-
2,3,5,10,11,11a-hexahydro-10-[2-(trimethylsilyl)ethoxymethyl]-
5,11-dioxo-1H-pyrrolo[2,1-c][1,4-]benzodiazepine (6)

A solution of 5 (6.50 g, 15.99 mmol) in anhydrous DMF (27.5 mL)
35 was added dropwise to a stirred suspension of NaH (0.422 g, 0.704

g of a 60 % dispersion in mineral oil, 18.34 mmol) at 0°C and the
reaction mixture was allowed to stir for 30 minutes. A solution
of SEM chloride (3.11 mL, 2.93 g, 17.59 mmol) in anhydrous DMF (5
mL) was added dropwise to the stirred reaction mixture at 0°C and
5   allowed to stir at room temperature for 16 hours. The reaction
mixture was poured into water (200 mL) to afford a white
precipitate, which was extracted with diethyl ether (4 x 300 mL).
The organic layer was washed with water (2 x 50 mL), brine (2 x
50 mL) and dried over anhydrous $MgSO_4$. Filtration and evaporation
10 of the solvent *in vacuo* afforded the crude product, which was
purified by flash column chromatography using an 80:20 mixture of
petroleum ether:EtOAc as eluent. Pure fractions were combined
and evaporated *in vacuo* to afford the product (**6**) as a yellow oil
(7.01 g, 13.1 mmol, 82 %). ^1H NMR (270 MHz, $CDCl_3$) δ 7.35 (s, 1H,
15 ArH), 7.24 (s, 1H, ArH), 5.52 (d, 2H, *J* = 9.89 Hz, SEM amino
acetal CH_2), 4.65 (d, 2H, *J* = 9.90 Hz, SEM amino acetal CH_2),
4.61-4.56 (m, 1H, *2*), 4.23 (dd, 1H, *J* = 4.40 Hz, 8.24 Hz, 11a),
3.94 (s, 3H, OMe), 3.92 (s, 3H, OMe), 3.68 (m, 4H, SEM 1= CH_2 +
3), 2.86 (m, 1H, *1*), 2.02 (m, 1H, *1*), 0.98 (t, 2H, *J* = 8.25 Hz,
20 SEM 2= CH_2), 0.88 (s, 9H, TBDMS t-Bu CH_3), 0.10 (s, 6H, 2 x TBDMS
$SiCH_3$), 0.03 (s, 9H, 3 x SEM $SiCH_3$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ
170.0, 165.6, 151.8, 147.1, 133.9, 121.5, 111.2, 105.5, 78.1,
69.6, 67.0, 56.5, 56.2, 56.1, 53.6, 35.5, 25.7, 18.4, -1.3, -4.8.

25 *(11aS)-6,7-dimethoxy-2(R)-hydroxy-2,3,5,10,11,11a-hexahydro-10-
[2-(trimethylsilyl)ethoxymethyl]-5,11-dioxo-1H-pyrrolo[2,1-
c][1,4-]benzodiazepine (7)*

A solution of 1 N TBAF in THF (19.58 mL, 19.58 mmol) was added to
a stirred solution of **6** (7.0 g, 13.05 mmol) in THF (50 mL). The
30 reaction mixture was allowed to stir at room temperature for 2
hours and diluted with DCM (200 mL), washed with water (2 x 200
mL), brine (2 x 200 mL) and dried over anhydrous $MgSO_4$.
Filtration and removal of excess solvent afforded the crude
product, which was purified by flash column chromatography using
35 50:50 petroleum ether:EtOAc as eluent. Evaporation *in vacuo* of
the pure fractions afforded the product (**7**) (5.9 g). ^1H NMR (270

MHz, CDCl₃) δ 7.30 (s, 1H, ArH), 7.24 (s, 1H, ArH), 5.52 (d, 1H, J = 9.9 Hz, SEM amino acetal CH₂), 4.68-4.64 (m, 2H, SEM amino acetal CH₂ + 2), 4.30 (dd, 1H, J = 5.86, 8.24 Hz), 3.91 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.87-3.51 (m, 4H, SEM 1= CH₂ + 3), 2.95

5   (dt, 1H, J = 5.31, 13.56 Hz, 1), 2.17-2.08 (m, 1H, 1), 1.02-0.93 (m, 2H, SEM 2= CH₂), 0.03 (s, 9H, 3 x SiCH₃); ¹³C NMR (67.8 MHz, CDCl₃) δ 169.7, 165.9, 151.9, 147.1, 134.0, 121.1, 111.2, 105.5, 78.2, 69.1, 67.1, 56.5, 56.1, 53.9, 34.9, 18.4, -1.3.

10   *(11aS)-6,7-dimethoxy-2,3,5,10,11,11a-hexahydro-10-[2-(trimethylsilyl)ethoxymethyl]-2,5,11-trioxo-1H-pyrrolo[2,1-c][1,4-]benzodiazepine (8)*

Anhydrous DMSO (3.28 g, 41.94 mmol) in dry DCM (20 mL) was added dropwise over 5 minutes to a stirred solution of oxalyl chloride

15   (10.48 mL of a 2 N solution in DCM, 20.97 mmol) under a nitrogen atmosphere at -50°C. After stirring for 5minutess, a solution 7 (5.90 g, 13.98 mmol), in dry DCM (45 mL) was added dropwise over 45 minutes to the reaction mixture, which was then stirred for a further 45 minutes at -50°C. TEA (9.89 g; 97.87 mmol) was added

20   dropwise to the mixture over 15 minutes followed by stirring for a further 15 minutes. The reaction mixture was left to warm to room temperature, diluted with H₂O (150 mL) and DCM (100 mL). The organic phase was washed with 1 N HCl (2 x 100 mL), water (2 x 100 mL), brine (2 x 100 mL) and dried over MgSO₄. Filtration and

25   evaporation afforded the crude product (8), which was purified by flash column chromatography using 50:50 petroleum ether (40-60°):EtOAc as eluent. Evaporation of the pure fractions *in vacuo* afforded the product (4.33 g, 10.3 mmol, 74 %). ¹H NMR (270 MHz, CDCl₃) δ 7.30 (s, 1H, ArH), 7.24 (s, 1H, ArH), 5.60 (d, 1H, J =

30   9.89 Hz, SEM amino acetal CH₂), 4.69 (d, 1H, J = 9.89 Hz, SEM amino acetal CH₂), 4.62 (dd, 1H, J = 9.89, 3.12 Hz, 11a), 4.26-4.19 (m, 1H, 3), 3.95 (s, 3H, OMe), 3.94 (s, 3H, OMe), 3.81-3.49 (m, 4H, SEM 1= CH₂ + 1 + 3), 2.82-2.71 (m, 1H, 1), 0.95 (t, 2H, J = 2.01 Hz, SEM 2= CH₂), -0.04 (s, 9H, SEM CH₃); ¹³C NMR (67.8 MHz,

35   CDCl₃) δ 206.8, 168.8, 165.9, 152.4, 147.5, 134.0, 120.4, 111.1, 105.6, 78.2, 67.2, 56.2, 54.8, 52.3, 37.3, 18.3, -1.3.

*(11aS)-5,10,11,11a-tetrahydro-7,8-dimethoxy-10-[2-
(trimethylsilyl)ethoxymethyl]-2-
[[(trifluoromethyl)sulphonyl]oxy]-5,11-dioxo-1H-pyrrolo[2,1-
c][1,4]benzodiazepine (9)*

Anhydrous pyridine (0.46 mL, 0.452 g, 5.73 mmol) was added in one
portion to a vigorously stirred solution of **8** (2.0 g, 4.77 mmol)
in anhydrous DCM (100 mL) and the mixture left to stir for 10
minutes at room temperature. Anhydrous triflic anhydride (1.25
mL, 1.48 g, 5.25 mmol) was added quickly, in one portion, and the
reaction mixture was allowed to stir at room temperature for 4.5
hours. The darkened, homogenous reaction mixture was poured into
cold saturated $NaHCO_3$ (200 mL) and the mixture was extracted with
DCM (3 x 50 mL). The organic layers were combined, washed with
water (2 x 200 mL), brine (2 x 200 mL) and dried over anhydrous
$MgSO_4$. Filtration and evaporation afforded the crude product,
which was purified by flash column chromatography using 80:20
petroleum ether:EtOAc as eluent. Evaporation of the pure
fractions *in vacuo* afforded the product (**9**) as a yellow oil (1.79
g, 3.25 mmol, 68 %). ^1H NMR (270 MHz, $CDCl_3$) δ 7.29 (s, 1H, ArH),
7.23 (s, 1H, ArH), 7.15 (t, 1H, *J* = 2.01 Hz, *H3*), 5.53 (d, 1H, *J*
= 10.07 Hz, SEM amino acetal CH_2), 4.68 (d, 1H, *J* = 9.89 Hz, SEM
amino acetal CH_2).

44

Example 1: Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-napthyl)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (11)
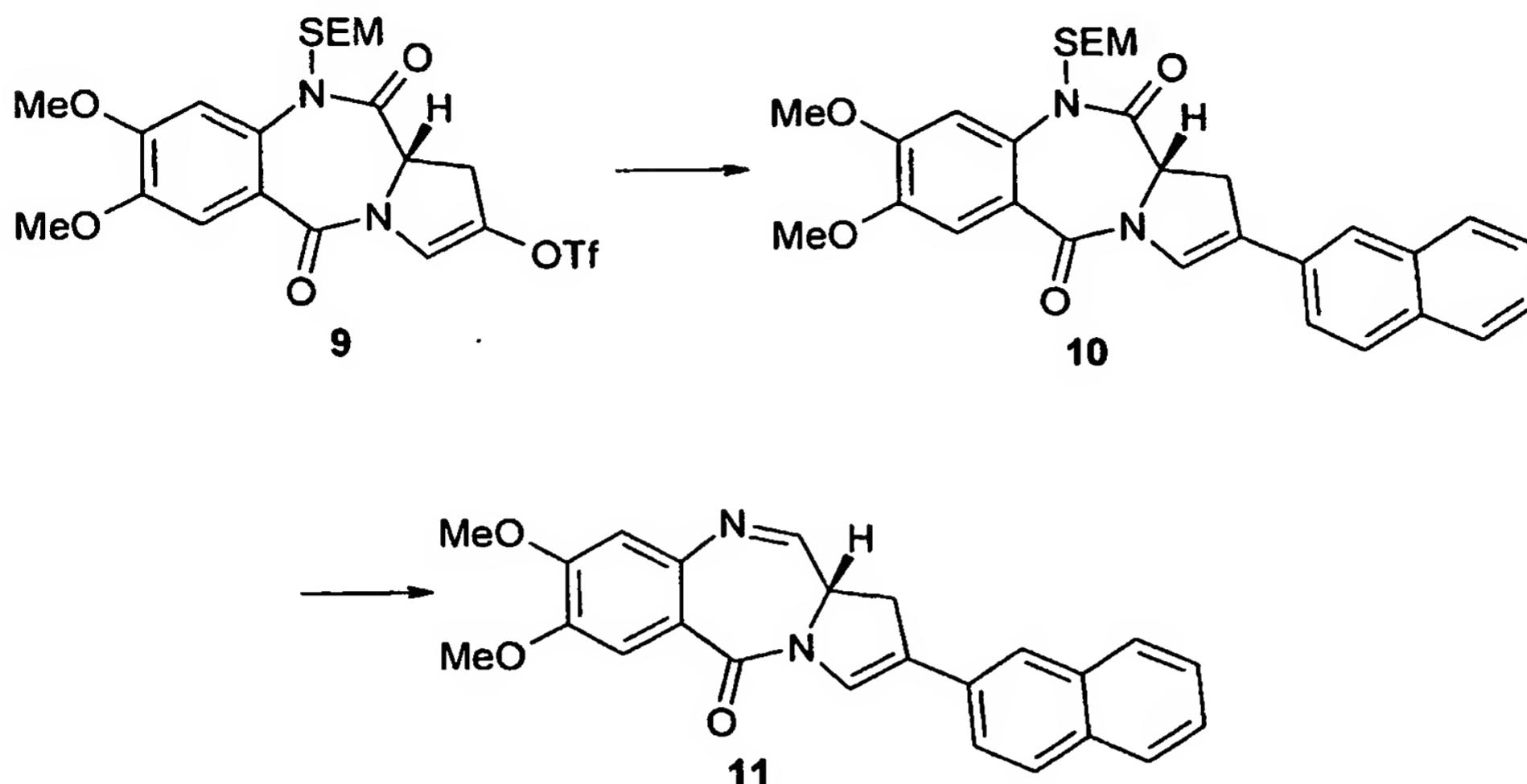
(a) *2-(2-napthyl)-7,8-dimethoxy-10-(2-trimethylsilanyl-*
5   *ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-*
5-one (10)

Sodium carbonate (300 mg, 2.83 mmol), 2-napthaleneboronic acid (171 mg, 0.99 mmol) and tetrakis(triphenylphosphine)palladium(0)(30 mg) were added to a
10 solution of **9** (517 mg, 0.93 mmol) in ethanol/water/benzene (20/20/20 mL) and stirred at room temperature for 96 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by flash
15 column chromatography using 70% ethyl acetate in hexane as eluent to give the pure product **10** as a yellow oil.

^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.85-7.71 (m, 4H, Ar-H x 4), 7.67-7.54 (m, 2H, H-3, Ar-H x 1), 7,52-7.39 (m, 3H, H-6, Ar-H x 2), 7.33-7.27 (s, 1H, H-9), 5.58 (d, 1H, J = 9.93 Hz, N-CH$_2$-OSEM x 1),
20 4.79-4.66 (m, 2H, N-CH$_2$-OSEM x 1, H-11a), 4.11 (d, 1H, J = 16.28 Hz, H-1 x 1), 3.97 & 3.95 (2s, 6H, 7- & 8- MeO), 3.90-3.65 (m, 2H, O-CH$_2$-SEM), 3.28 (dd, 1H, J = 10.92, 16.23 Hz, H-1 x 1), 1.00 (t, 2H, J = 8.23 Hz, CH$_2$-SEM), 0.04 (s, 9H, SEM-Si(CH$_3$)$_3$).

25

(b) *(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-napthyl)-5H-*
pyrrolo[2,1-c][1,4]benzodiazepin-5-one **(11)**

Solid sodium tetraborohydride (654 mg, 17.2 mmol) was added in
three portions at two hourly intervals to a stirred solution of

5 **10** (971 mg, 1.8 mmol) in a mixture of anhydrous ethanol (19 mL)
and anhydrous THF (37 mL) and left to stir at room temperature
for 24 hours. The reaction mixture was diluted with water (50
mL) and extracted with ethyl acetate (3x 50 mL). The organic
layers were evaporated *in vacuo*. The crude product was treated

10 with ethanol (50 mL), water (30 mL) and silica gel (18.3 g) and
left to stir at room temperature for 24 hours. The reaction
mixture was filtered and the product extracted with ethyl acetate
(3 x 50 mL). The organic layers were combined and washed with
brine (20 mL) and dried over anhydrous magnesium sulphate.

15 Filtration and evaporation *in vacuo* afforded the crude product,
which was purified using flash column chromatography with a 70-
50 % hexane in ethyl acetate as eluent. Pure fractions were
combined and evaporated *in vacuo* to afford the pure product **11** as
a yellow powder (151 mg, 0.39 mmol, 22 % yield).

20 ^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.9 (d, 1H, J = 4.0 Hz, H11), 7.86-
7.40 (m, 8H, H3, H6, 6 x Ar-H), 6.85 (s, 1H, H9), 4.48 (dt, 1H, J
= 4.9, 11.2 Hz, H11a), 3.98 & 3.95 (2s, 6H, 7- & 8-MeO), 3.80-
3.46 (m, 2H, 2 x H1).

46

Example 2: Synthesis of (11a*S*)-1,11a-dihydro-7,8-dimethoxy-2-(4-tert-butylbenzene)-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (13)
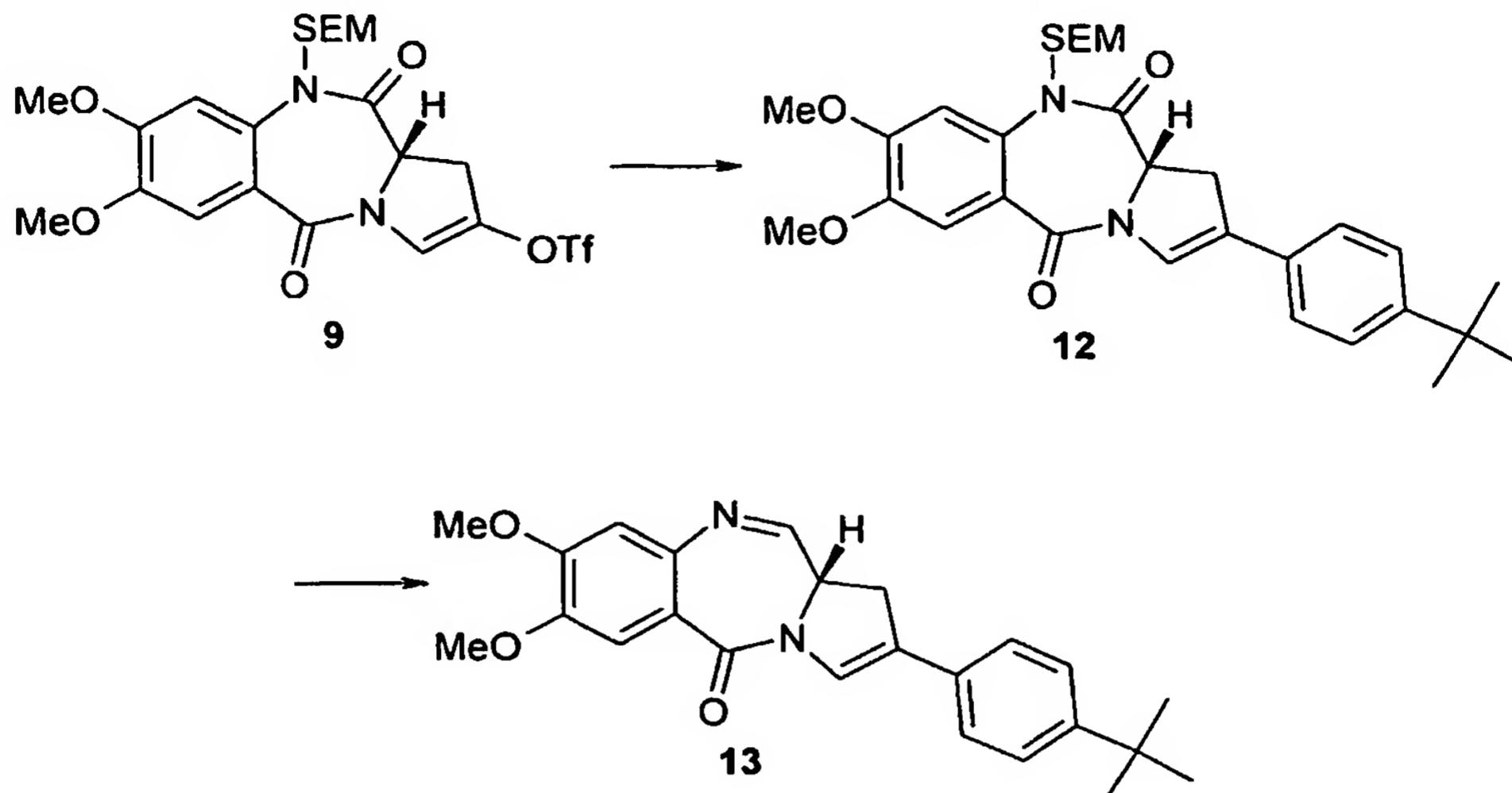
(a) *2-(4-tert-butylbenzene)-7,8-dimethoxy-10-(2-trimethylsilanyl-*

5   *ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-*
    5-one (12)

Sodium carbonate (302 mg, 2.85 mmol), 4-*tert*-butylbenzeneboronic acid (177 mg, 0.99 mmol) and tetrakis(triphenylphosphine)palladium(0) (30 mg) were added to a

10 solution of **9** (502 mg, 0.91 mmol) in ethanol/water/benzene (20/20/20 mL) and stirred at room temperature for 41 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated

15 flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product **12** (408 mg, 0.76 mmol, 84 % yield).

^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.45-7.29 (m, 7H, H-6 & H-3 & H-9 & Ar-H x 4), 5.56 (d, 1H, *J* = 9.97 Hz, N-CH$_2$-OSEM x 1), 4.78-4.55

20 (m, 2H, N-CH$_2$-OSEM x 1, H-11a), 4.04-3.60 (m, .9H, 7- & 8- MeO and H-1 x 1 & O-CH$_2$-SEM), 3.16 (dd, 1H, *J* = 10.35, 15.52 Hz, H-1 x 1), 1.38-1.21 (multiple singlets not all Me groups equivalent, 9H, tert-Bu (CH$_3$)$_3$), 1.04-0.80 (m, 2H, CH$_2$-SEM), 0.09-(-0.04) (multiple singlets not all Me groups equivalent, 9H, SEM-Si(CH$_3$)$_3$

25

(b) *(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-tert-butylbenzene)-*
5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (13)

Sodium tetraborohydride (531mg, 14.0 mmol) was added in three
portions at 0, 5 and 30 hours to a stirred solution of **12** (398

5   mg, 0.74 mmol) in a mixture of anhydrous ethanol (7.5 mL) and
anhydrous THF (15 mL). The reaction mixture was allowed to stir
at room temperature for 44 hours. The reaction mixture was
diluted with water (50 mL) and extracted with ethyl acetate (3x
50 mL). The organic layers were evaporated *in vacuo*. The crude

10   product was treated with ethanol (50 mL), water (25 mL) and
silica gel (7.5 g) and left to stir at room temperature for 24
hours. The reaction mixture was filtered and the product
extracted with ethyl acetate (3 x 50 mL). The organic layers
were combined and washed with brine (20 mL) and dried over

15   anhydrous magnesium sulphate. Filtration and evaporation *in*
vacuo afforded the crude product, which was purified using flash
column chromatography with a 70%-50% hexane in ethyl acetate as
eluent. Pure fractions were combined and evaporated *in vacuo* to
afford the pure product **13** as yellow glassy solid (193 mg, 0.49

20   mmol, 67 % yield).

^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.89 (d, 1H, J = 3.94 Hz, H11), 7.58-
7.17 (m, 6H, H3,H6, 4 x Ar-H), 6.84 (s, 1H, H9), 4.49-4.35 (m,
1H, H11a), 4.05- 3.51 (m, 7H, 7- & 8-MeO, H1), 3.49- 3.33 (m, 1H,
H1), 1.34 (s, 9H, Ar-tBu).

25

Example 3: Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-chlorobenzene)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (15)
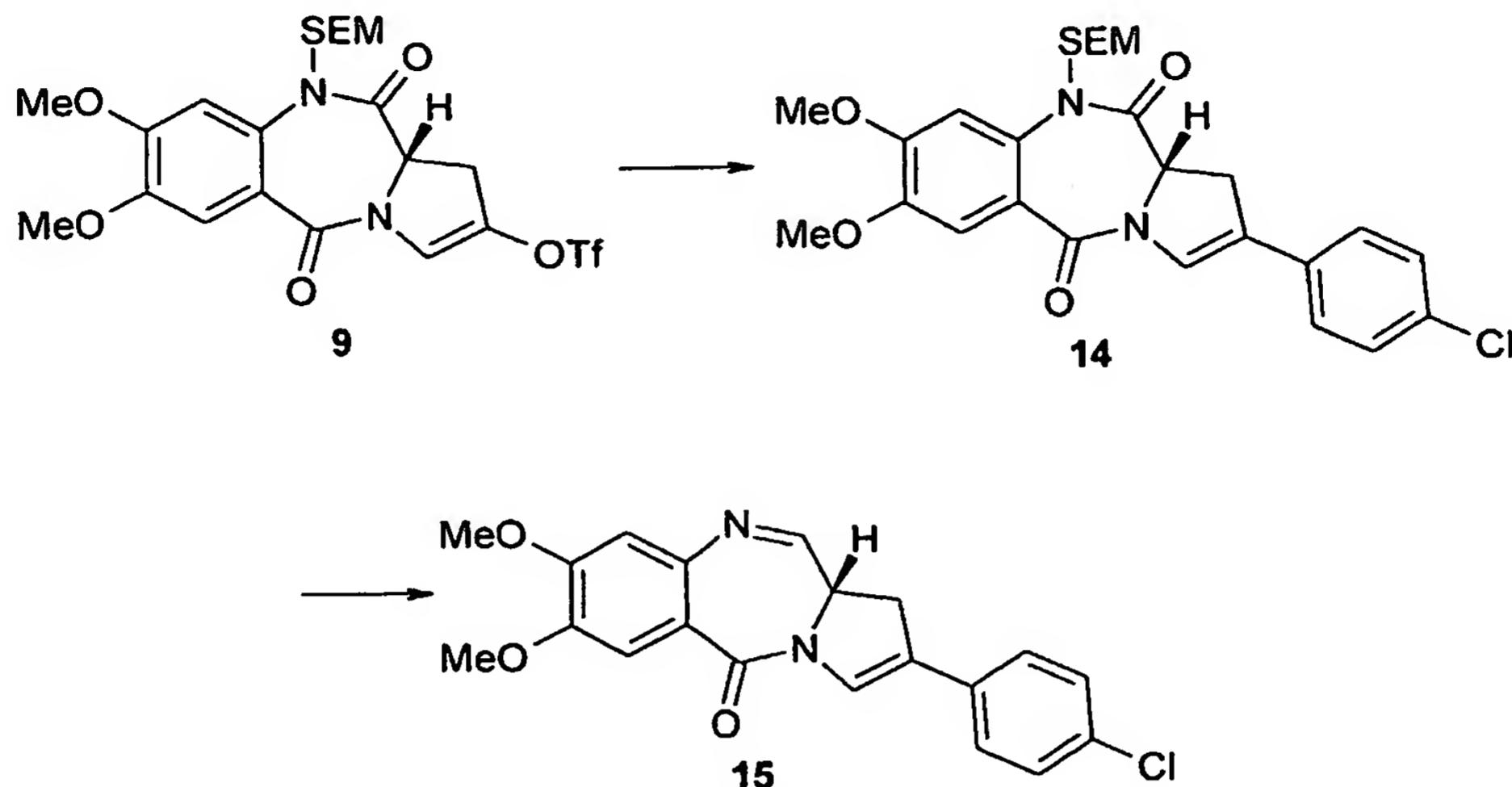
(a) *2-(4-chlorobenzene)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (14)*

Sodium carbonate (300 mg, 2.83 mmol), chlorobenzeneboronic acid (155.28 mg, 1.00 mmol) and tetrakis(triphenylphosphine)palladium(0)(30 mg) were added to a solution of **9** (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and stirred at room temperature for 46 hours at which point additional chlorobenzeneboronic acid (155 mg, 0.99 mmol) and tetrakis(triphenylphosphine)palladium(0)(30 mg) were added. The solution was stirred for a further 44 hours and then diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL). The solution was dried over magnesium sulphate and purified by flash column chromatography using 70% ethyl acetate in hexane as eluent to give the pure product **14** as a brown oil (411 mg, 0.86 mmol, 95 % yield).

^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.46-7.22 (m, 7H, H-3 & H-6 & Ar-H x 4 & H-9), 5.55 (d, 1H J = 9.92 Hz, N-CH$_2$-OSEM x 1), 4.77-4.61 (m, 2H, N-CH$_2$-OSEM x 1 & H-11a), 4.01-3.87 (m, 7H, H-1 x 1 and 7- & 8-MeO), 3.87-3.63 (m, 2H, O-CH$_2$-SEM), 3.13 (ddd, 1H, J = 2.09, 10.56, 16.15 Hz, H-1 x 1), 0.98 (t, 2H, J = 8.20 Hz, CH$_2$-SEM), 0.03 (s, 9H, SEM-Si(CH$_3$)$_3$).

(b) *(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-chlorobenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one* **(15)**

Sodium tetraborohydride (531mg, 14 mmol) was added in three

5   portions at 0, 5 and 12 hours to a stirred solution of **14** (386
mg, 0.75 mmol) in a mixture of anhydrous ethanol (7.5 mL) and
anhydrous THF (15 mL) and left to stir at room temperature for 24
hours. The reaction mixture was diluted with water (50 mL) and
extracted with ethyl acetate (3x 50 mL). The organic layers were

10 evaporated *in vacuo*. The crude product was treated with ethanol
(50 mL), water (25 mL) and silica gel (7.5 g) and left to stir at
room temperature for 24 hours. The reaction mixture was filtered
and the product extracted with ethyl acetate (3 x 50 mL). The
organic layers were combined and washed with brine (20 mL) and

15 dried over anhydrous magnesium sulphate. Filtration and
evaporation *in vacuo* afforded the crude product, which was
purified using flash column chromatography with a 70%-50% hexane
in ethyl acetate as eluent. Pure fractions were combined and
evaporated *in vacuo* to afford the pure product **15** as a yellow

20 solid (97.5 mg, 0.26 mmol, 35 % yield).

^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.90 (d, 1H, J = 3.9 Hz, H11), 7.53 (s,
1H, H6), 7.50 (broad s, 1H, H3), 7.32 (s, 4H, 4 x Ar-H), 6.84 (s,
1H H9), 4.44 (ddd, 1H, J = 4.1, 5.3, 11.4 Hz, H11a), 3.97 & 3.95
(2s, 6H, 7- & 8-MeO), 3.58 (ddd, 1H, J = 1.98, 11.6, 16.3 Hz,

25 H1), 3.4 (ddd, 1H, J = 1.7, 5.4, 16.3 Hz, H1).

Example 4: Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-fluorobenzene)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (17)
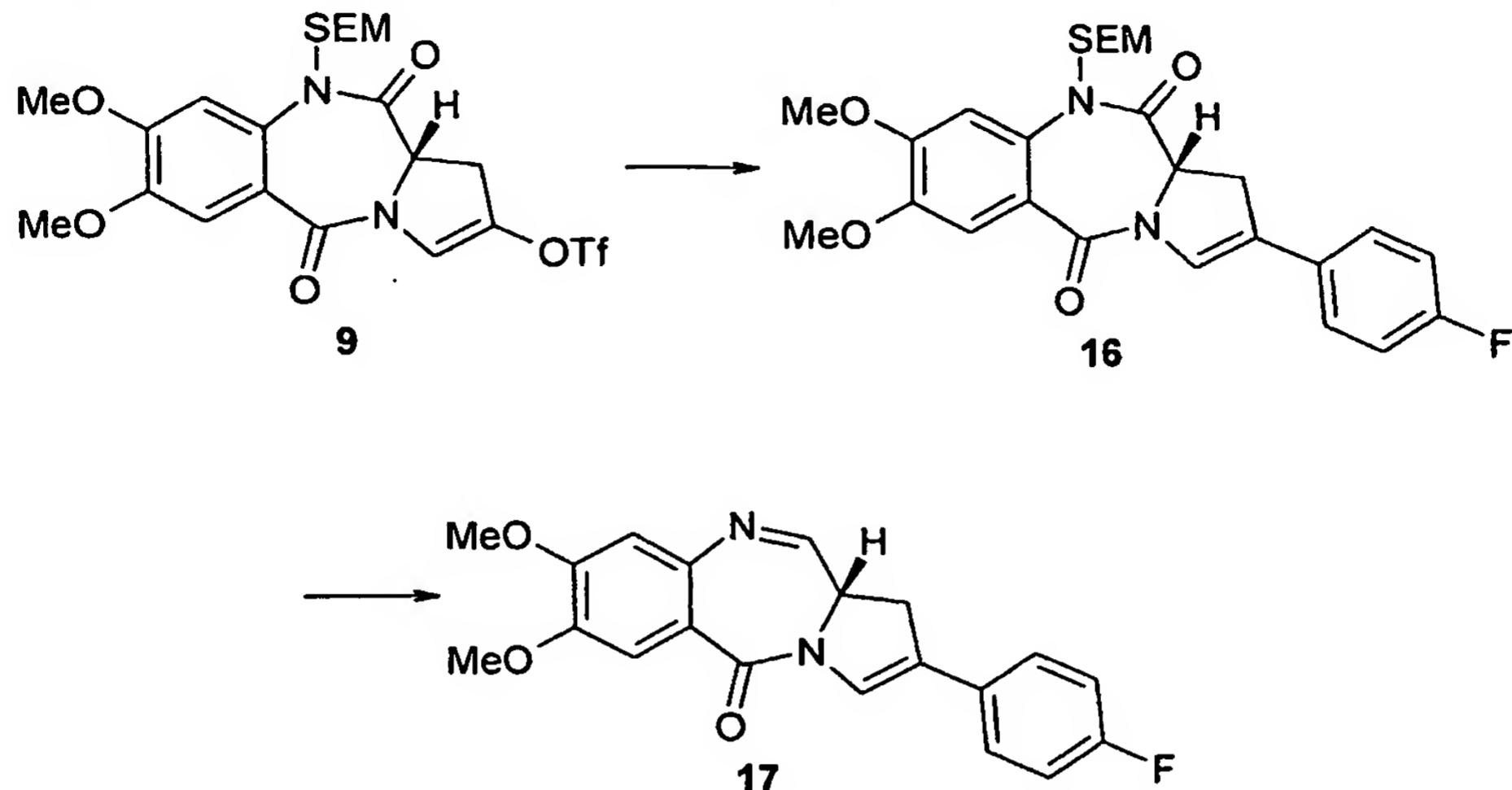
(a) *2-(4-fluorobenzene)-7,8-dimethoxy-10-(2-trimethylsilanyl-*
5 *ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-*
5-one (16)

Sodium carbonate (308 mg, 2.91 mmol), 4-fluorobenzeneboronic acid (139 mg, 0.99 mmol) and tetrakis(triphenylphosphine) palladium(0) (30 mg) were added to a solution of **9** (500 mg, 0.90 mmol) in
10 ethanol/water/benzene (20/20/20 mL) and stirred at room temperature for 93 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 –
15 50 % hexane in ethyl acetate gradient system as eluent to give the pure product **16** as a yellow oil (441 mg, 0.88 mmol, 99 % yield).

^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.45-7.32 (m, 5H, H-6 & H-3 & Ar-H x 2), 7.27 (s, 1H, H-9), 7.04 (t, 2H, Ar-H x 2), 5.56 (d, 1H, J =
20 9.80 Hz, N-CH$_2$-OSEM x 1), 4.72 (d, 1H, J = 9.49 Hz, N-CH$_2$-OSEM x 1), 4.65 (dd, 1H, J = 3.28, 10.17 Hz, H-11a), 4.04-3.88 (m, 7H, 7- & 8- MeO and H-1 x 1), 3.88- 3.64 (m, 2H, O-CH$_2$-SEM), 3.14 (ddd, 1H, J = 2.26, 10.57, 16.25 Hz, H-1 x 1), 0.99 (t, 2H, J = 8.33 Hz, CH$_2$-SEM), 0.03 (s, 9H, SEM-Si(CH$_3$)$_3$).

25

(b) *(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(4-fluorobenzene)-5H-*
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (17)

Sodium tetraborohydride (201mg, 5.3 mmol) was added in two
portions at 0 and 5 hours to a stirred solution of **16** (429 mg,
5   0.861 mmol) in a mixture of anhydrous ethanol (8.6 mL) and
anhydrous THF (17.2 mL). The reaction mixture was allowed to
stir at room temperature for 24 hours. The reaction mixture was
diluted with water (50mL) and extracted with ethyl acetate (3x
50mL). The organic layers were evaporated *in vacuo.* The crude
10 product was treated with ethanol (50 mL), water (25 mL) and
silica gel (8.6 g) and left to stir at room temperature for 24
hours. The reaction mixture was filtered and the product
extracted with ethyl acetate (3 x 50 mL). The organic layers
were combined and washed with brine (20 mL) and dried over
15 anhydrous magnesium sulphate. Filtration and evaporation *in*
vacuo afforded the crude product, which was purified using flash
column chromatography with a 70%-50% hexane in ethyl acetate as
eluent. Pure fractions were combined and evaporated *in vacuo* to
afford the pure product **17** as a yellow glassy solid (139 mg, 0.39
20 mmol, 46 % yield).

^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.90 (d, 1H, J = 3.9 Hz, H11), 7.53 (s,
1H, H6), 7.45 (s, 1H, H3), 7.40-7.20 (m, 1H, 1 x Ar-H), 7.10-6.90
(m, 3H, 3 x Ar-H), 6.84 (s, 1H, H9), 4.43 (ddd, 1H, J = 4.1, 5.4,
11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8-MeO), 3.69- 3.50 (m,
25 1H, H1), 3.39 (ddd, 1H, J = 1.5, 5.3, 16.3 Hz, H1).

Example 5: Synthesis of (11a*S*)-1,11a-dihydro-7,8-dimethoxy-2-(2-methylbenzene)-5*H*-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (19)

9 → **18**

→ **19**
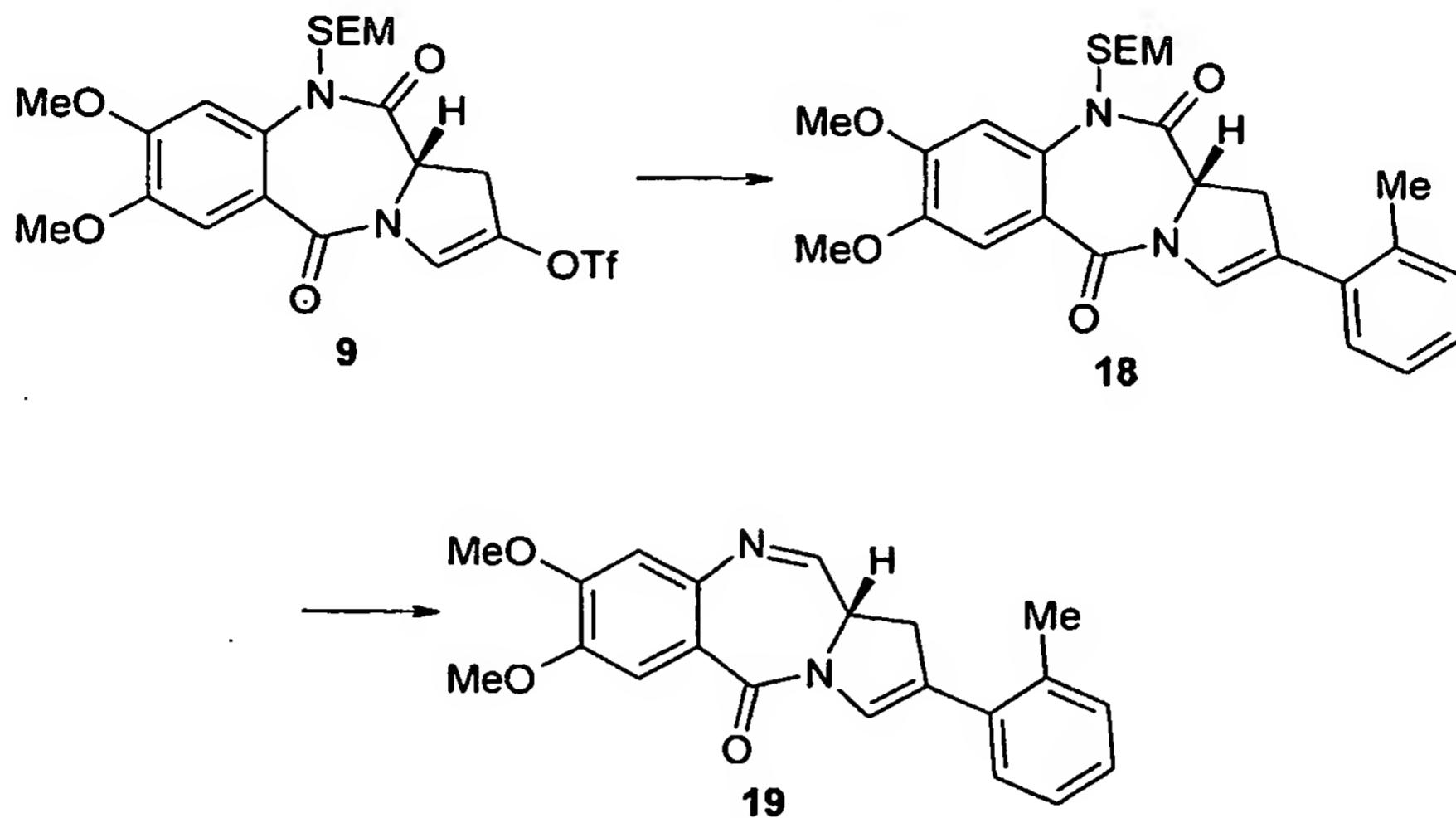
(a) *2-(2-methylbenzene)-7,8-dimethoxy-10-(2-trimethylsilanyl-*
ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-
5-one (18)

Sodium carbonate (300 mg, 2.83 mmol), 2-methylbenzeneboronic acid (135 mg, 0.99 mmol) and tetrakis(triphenylphosphine)palladium(0) (30 mg) were added to a solution of **9** (500 mg, 0.90 mmol) in ethanol/water/benzene (20/20/20 mL) and stirred at room temperature for 20 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product **18** as a yellow oil (439 mg, 0.89 mmol, 98 % yield).

^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.44-7.09 (m, 7H, H-6 & H-3 & H-9 & Ar-H x 4), 5.59 (d, 1H, J = 9.97 Hz, N-CH$_2$-OSEM x 1), 4.78-4.50 (m, 2H, N-CH$_2$-OSEM x 1, H-11a), 4.06-3.60 (m, 9H, 7- & 8- MeO, O-CH$_2$-SEM, H-1 x 1), 3.22 (dd, 1H, J = 10.23, 16.31 Hz, H-1 x 1), 2.48 (Broad s, 3H, Ar-2-Me), 1.06-0.79 (m, 2H, CH$_2$-SEM), 0.03 (broad s, 9H, SEM-Si(CH$_3$)$_3$).

53

(b) *(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-methylbenzene)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one* **(19)**

Sodium tetraborohydride (500 mg, 13.2 mmol) was added in three portions at 0, 5 and 12 hours to a stirred solution of **18** (429

5   mg, 0.87 mmol) in a mixture of anhydrous ethanol (8.5 mL) and anhydrous THF (17.5 mL). The reaction mixture was allowed to stir at room temperature for 24 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated *in vacuo*. The crude

10 product was treated with ethanol (50 mL), ice water (25 mL) and silica gel (8.5 g) and left to stir at room temperature for 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over

15 anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated *in vacuo* to afford the pure product **19** as a yellow solid (74 mg, 0.21 mmol,

20 25 % yield).

^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.92 (d, 1H, J = 3.9 Hz, H11), 7.55 (s, 1H, H6), 7.34- 7.14 (m, 5H, H3, 4 x Ar-H), 6.85 (s, 1H, H9), 4.39 (dt, 1H, J = 4.7, 11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8-MeO), 3.78- 3.59 (m, 1H, H1), 3.47 (dd, 1H, J = 5.2, 16.2 Hz,

25 H1), 2.49 (s, 3H, Ar-CH$_3$).

Example 6: Synthesis of (11a*S*)-1,11a-dihydro-7,8-dimethoxy-2-(2-methylbenzene)-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (21)
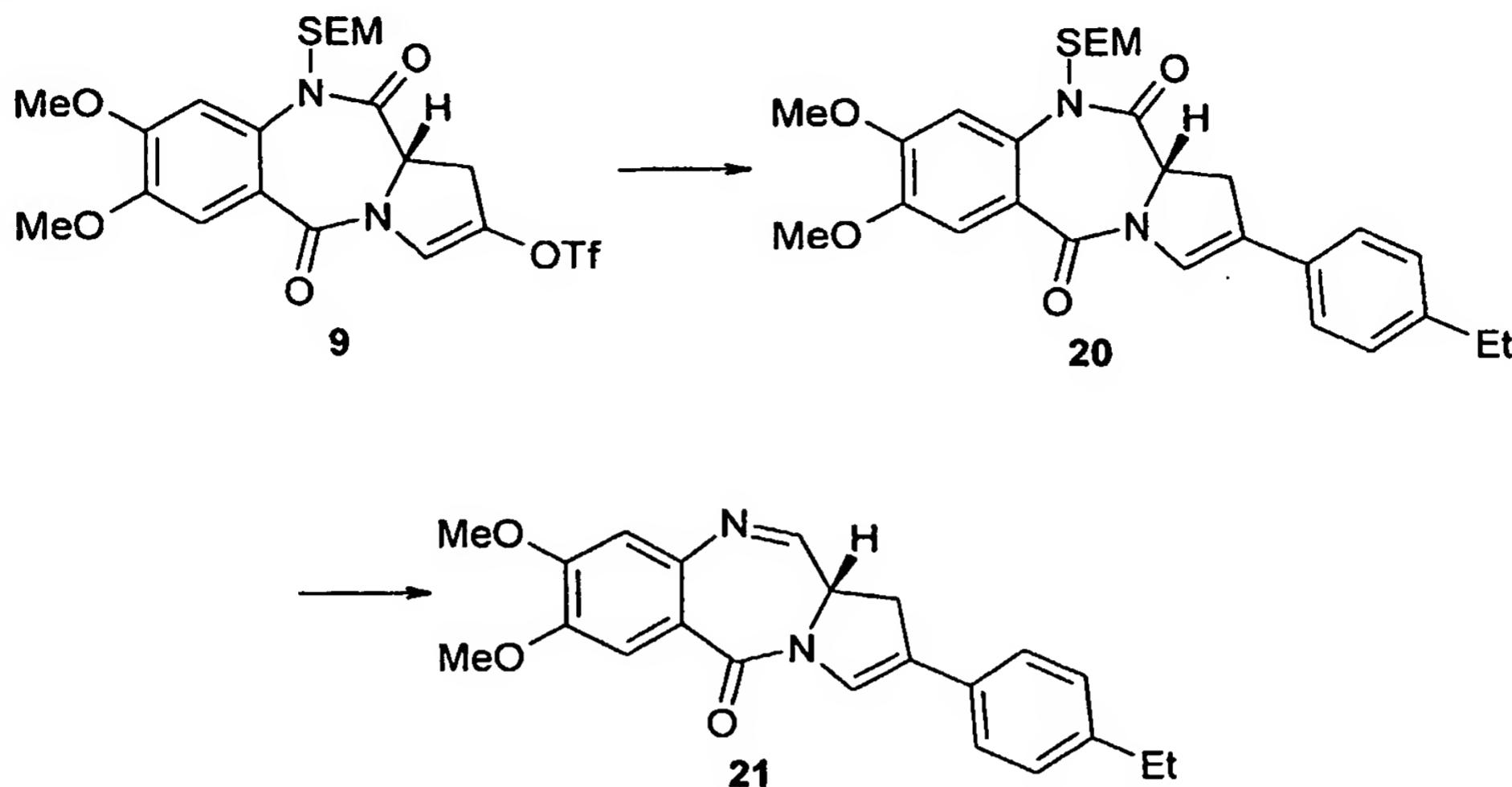
(a) 2-(4-ethylbenzene)-7,8-dimethoxy-10-(2-trimethylsilanyl-

5   *ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (20)*

Sodium carbonate (302 mg, 2.85 mmol), 4-ethylbenzeneboronic acid (149 mg, 0.993 mmol) and tetrakis(triphenylphosphine) palladium(0) (30 mg) were added to a solution of **9** (501 mg, 0.91

10   mmol) in ethanol/water/benzene (20/20/20 mL) and stirred at room temperature for 41 hours. The reaction mixture was diluted with ethyl acetate (220 mL) and washed with water (50 mL) and brine (50 mL) and dried over magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 –

15   50 % hexane in ethyl acetate gradient system as eluent to give the pure product **20** as a yellow solid (342 mg, 0.67 mmol, 74 % yield).

^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.46-7.15 (m, 7H, H-6 & H-3 & H-9 & Ar-H x 4), 5.57 (d, 1H, *J* = 9.88 Hz, N-CH$_2$-OSEM x 1), 4.72 (d, 1H,

20   *J* = 9.95 Hz, N-CH$_2$-OSEM x 1), 4.65 (dd, 1H, *J* = 3.52, 10.85 Hz, H-11a), 4.03-3.88 (m, 7H, 7- & 8- MeO and H-1 x 1), 3.88- 3.64 (m, 2H, O-CH$_2$-SEM), 3.16 (dd, 1H, *J* = 10.84, 16.51 Hz, H-1 x 1), 2.66 (q, 2H, *J* = 7.68 Hz, Ar-CH$_2$-Me x 2), 1.24 (t, 3H, *J* = 7.57 Hz, ArCH$_2$-*CH$_3$*), 0.99 (t, 2H, *J* = 8.29 Hz, CH$_2$-SEM), 0.04 (s, 9H, SEM-

25   Si(CH$_3$)$_3$).

55

(b)   (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-methylbenzene)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one (21)

Sodium tetraborohydride (500 mg, 13.2 mmol) was added in three

5   portions at 0, 5 and 12 hours to a stirred solution of **20** (429
mg, 0.87 mmol) in a mixture of anhydrous ethanol (8.5 mL) and
anhydrous THF (17.5 mL). The reaction mixture was allowed to stir
at room temperature for 24 hours. The reaction mixture was
diluted with water (50 mL) and extracted with ethyl acetate (3 x

10 50 mL). The organic layers were evaporated *in vacuo*. The crude
product was treated with ethanol (50 mL), ice water (25 mL) and
silica gel (8.5 g) and left to stir at room temperature for 24
hours. The reaction mixture was filtered and the product
extracted with ethyl acetate (3 x 50 mL). The organic layers

15 were combined and washed with brine (20 mL) and dried over
anhydrous magnesium sulphate. Filtration and evaporation *in*
vacuo afforded the crude product, which was purified using flash
column chromatography with a 70%-50% hexane in ethyl acetate as
eluent. Pure fractions were combined and evaporated *in vacuo* to

20 afford the pure product **21** as a yellow solid (74 mg, 0.21 mmol,
25 % yield).

^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.92 (d, 1H, J = 3.9 Hz, H11), 7.55 (s,
1H, H6), 7.34-7.14 (m, 5H, H3, 4 x Ar-H), 6.85 (s, 1H, H9), 4.39
(dt, 1H, J = 4.7, 11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8-

25 MeO), 3.78-3.59 (m, 1H, H1), 3.47 (dd, 1H, J = 5.2, 16.2 Hz, H1),
2.49 (s, 3H, Ar-CH$_3$).

56

Example 7: Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-thiophene)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (23)

9   **22**

23
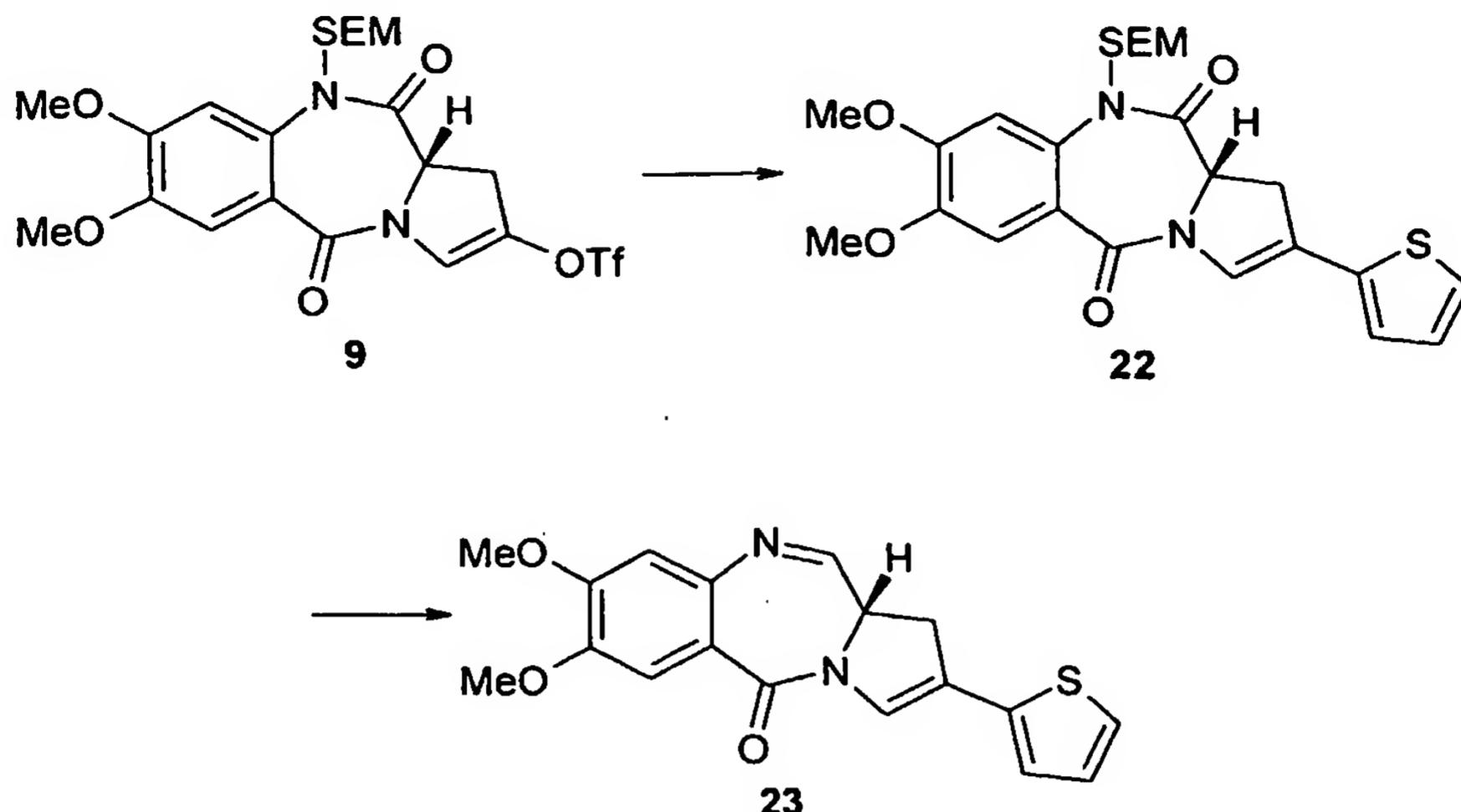
(a) 2-(2-thiophene)-7,8-dimethoxy-10-(2-trimethylsilanyl-

5 *ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-*
   5-one (22)

   Sodium carbonate (305 mg, 2.88 mmol), 2-thiopheneboronic acid
   (127 mg, 0.99 mmol) and tetrakis(triphenylphosphine) palladium(0)
   (30 mg) were added to a solution of **9** (500 mg, 0.90 mmol) in

10 ethanol/water/benzene (20/20/20 mL) and stirred at room
   temperature for 164.5 hours and refluxed at 80°C for a further 4
   hours. The reaction mixture was diluted with ethyl acetate (200
   mL) and washed with water (50 mL) and brine (50 mL) and dried
   over magnesium sulphate. The crude product was purified by

15 automated flash column chromatography using a 100 - 50 % hexane
   in ethyl acetate gradient system as eluent to give the pure
   product **22** as a yellow oil (353 mg, 0.73 mmol, 80 % yield).

   ^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.39 (s, 1H, H-6), 7.32-7.17 (m, 3H,
   H-3, H-9, Ar-H x 1), 7.06-6.94 (m, 2H, Ar-H x 2), 5.56 (d, 1H, J

20 = 9.97 Hz, N-CH$_2$-OSEM x 1), 4.71 (d, 1H, J = 9.91 Hz, N-CH$_2$-OSEM x
   1), 4.64 (dd, 1H, J = 3.51, 10.56 Hz, H-11a), 4.02-3.88 (m, 7H,
   7- & 8- MeO, H-1 x 1), 3.88-3.63 (m, 2H, O-CH$_2$-SEM), 3.18 (ddd,
   1H, J = 2.38, 10.76, 16.11 Hz, H-1 x 1), 0.99 (t, 2H, J = 8.24
   Hz, CH$_2$-SEM), 0.04 (s, 9H, SEM-Si(CH$_3$)$_3$).

25

57

(b) *(11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-thiophene)-5H-*
pyrrolo[2,1-c][1,4]benzodiazepin-5-one **(23)**

Sodium tetraborohydride (520mg, 13.7 mmol) was added in three
portions at 0, 7 and 14 hours to a stirred solution of **22** (333

5   mg, 0.69 mmol) in a mixture of anhydrous ethanol (6.9 mL) and
anhydrous THF (13.8 mL) and left to stir at room temperature for
30 hours. The reaction mixture was diluted with water (50 mL)
and extracted with ethyl acetate (3x 50 mL). The organic layers
were evaporated *in vacuo* and the crude product was treated with

10 ethanol (50 mL), water (25 mL) and silica gel (6.9 g) and left to
stir at room temperature for 24 hours. The reaction mixture was
filtered and the product extracted with ethyl acetate (3 x 50
mL). The organic layers were combined and washed with brine (20
mL) and dried over anhydrous magnesium sulphate. Filtration and

15 evaporation *in vacuo* afforded the crude product, which was
purified using flash column chromatography with a 70%-50% hexane
in ethyl acetate as eluent. Pure fractions were combined and
evaporated *in vacuo* to afford the pure product **23** as a yellow
solid (49.6 mg, 0.15 mmol, 21 % yield).

20 MS (ES) m/z(relative intensity): 389.2 (22), 373.2 (14), 341 (8),
194.3 (100).

Example 8: Synthesis of (11a*S*)-1,11a-dihydro-7,8-dimethoxy-2-(2-
furan)-5*H*-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (25)

9

24

25

25

58
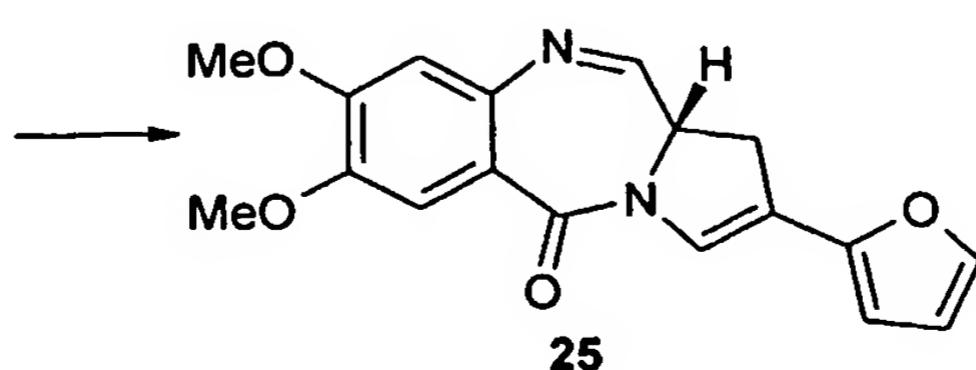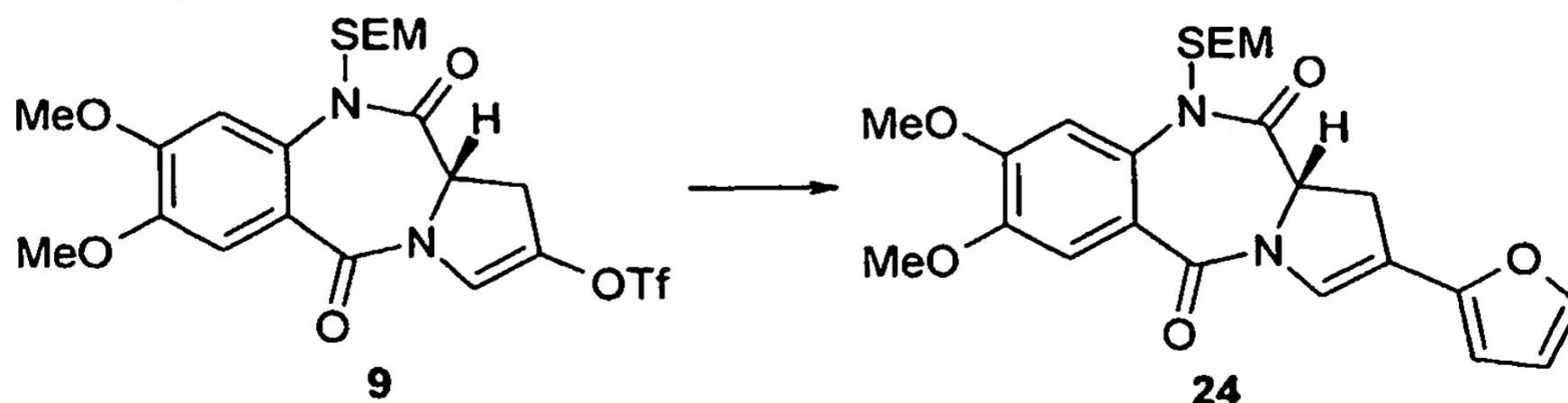
*(a) 2-(2-furan)-7,8-dimethoxy-10-(2-trimethylsilanyl-
ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-
5-one **(24)***

A catalytic amount of tetrakis triphenylphosphine palladium [0]

5   (5 mol%, 73 mg, 0.063 mmol) was added to a stirred mixture of
9(700 mg, 1.27 mmol), LiCl (161 mg, 3.8 mmol), and 2-
(tributylstannyl)furan (0.526 mL, 615 mg, 1.65 mmol) in anhydrous
THF (20 mL) and heated at reflux for 2½ hours. The cooled
reaction mixture was diluted with DCM (100 mL) and the mixture

10 washed with 10% aqueous ammonium hydroxide (100 mL). The organic
layer was washed with water (100 mL), brine (100 mL), and dried
over anhydrous $MgSO_4$. Filtration and evaporation of the solvent in
vacuo afforded the crude product, which was further purified by
flash chromatography using a 75:25 mixture of hexane:EtOAc as

15 eluent. Pure fractions were combined and evaporation of the
solvent *in vacuo* afforded the product **24** (484 mg, 1.03 mmol,
81%).

^{1}H NMR (CDCl$_3$) δ 7.35 (s, 2H), 7.27 (s, 1H),7.23 (d, J$_1$=1.5Hz,
1H), 6.37 (dd,J$_1$=1.7Hz, J$_2$=3.2Hz, 1H), 6.26 (d, J$_1$=3.2Hz), 5.52

20 (d, J=10Hz, 1H, SEM), 4.67 (d, J=10Hz, 1H, SEM), 4.58 (dd,
J$_1$=3.3Hz, J$_2$=10.6Hz, 1H, H11a), 3.91 (s, 3H, OMe), 3.90 (s, 3H,
OMe), 3.86-3.74 (m, 2H, 1SEM, 1H1), 3.69-3.63 (m, 1H, 1SEM), 3.05
(ddd, J$_1$=2.0 Hz, J$_2$=10.6Hz, J$_3$=15.8Hz, 1H1), 0.95 (m, 2H, SEM), 0
(s, 9H, SEM).

25 ^{13}C NMR (CDCl$_3$)δ 169.5, 163, 153.4, 150.6, 148.7, 143.7, 135,
122.6, 122.4, 117.7, 112.7, 108.6, 107.2, 79.7, 68.51, 58.75,
57.5, 31.8, 19.7, 0
IR 2952, 1689, 1640, 1606, 1518, 1453, 1430, 1358, 1276, 1248,
1209, 1102, 1069, 1010, 858, 836, 787, 757.

30 MS ES$^+$ 471.2 (M+H, 100%), 353.1 (65%).
[α]$_d$=+175°, c=20mg/10mL, T=18.6°C, CHCl$_3$.

*(b) (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2-furan)-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one **(25)***

35 Solid sodium tetraborohydride (NaBH$_4$, 95 mg, 2.51mmol) was added
in one portion to a rapidly stirred solution of **24** (400mg,
0.85mmol) in a mixture of anhydrous EtOH (3 mL) and anhydrous THF

59

(7 mL) at room temperature and allowed to stir for 4 hours. More
sodium tetraborohydride (60mg, 1.58 mmol) was added and the
mixture left to stir at room temperature overnight. The reaction
mixture was diluted with cold water (50 mL) and extracted with
5   EtOAc (2 x 30 mL). The organic layers were washed with brine (30
mL) and dried over anhydrous $MgSO_4$. Filtration and evaporation of
the solvent *in vacuo* afforded the crude product which was stirred
overnight with silica gel (1 g) in EtOH (10 mL) and water (5 mL).
The mixture was then filtered and excess EtOH was removed by
10 rotary evaporation. The remaining mixture was extracted with
EtOAc (2 x 20 mL). The organic layers were washed with brine (30
mL) and dried over anhydrous $MgSO_4$. Filtration and evaporation of
the solvent *in vacuo* afforded the crude product which was
purified by flash column chromatography eluting with a 60:40
15 mixture of hexane:EtOAc. Pure fractions were combined to afford
25 as a yellow solid (80 mg, 0.246 mmol, 29%).

^{1}H NMR ($CDCl_3$) δ 7.89 (d, J=4.0 Hz, 1H, H-11), 7.54 (s, 1H),7.42
(d, J=1.4Hz, 1H), 7.38 (s, 1H), 6.84 (s, 1H) 6.44 (dd, J_1=1.7Hz,
J_2=3.2Hz, 1H), 6.28 (d, J=3.2Hz, 1H), 4.41 (m, 1H, H11a), 3.98 (s,
20 3H, MeO), 3.96 (s, 3H, MeO), 3.50(m, 1H1), 3.35 (ddd, J_1=1.6Hz,
J_2=5.2Hz, J_2=16.2Hz, 1H, 1H1).

^{13}C NMR ($CDCl_3$) δ 163.8, 162.8, 153.4, 150.7, 149.3, 143.9, 141.8,
124.3, 120.5, 115.8, 113.1, 112.9, 111.3, 108.2, 57.7, 57.6,
55.2, 36.

25 IR 3117, 2936, 1626, 1601, 1559, 1508, 1450, 1426, 1377, 1344,
1265, 1215, 1102, 1072, 1039, 1007, 956, 914, 882, 778, 730.

MS ES$^+$ 325.1 (M+H, 100%).

$[\alpha]_d$=+457°, c=15mg/10mL, T=20°C. $CHCl_3$

30

Example 9: Synthesis of (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2,6-dimethylbenzene)-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (27)
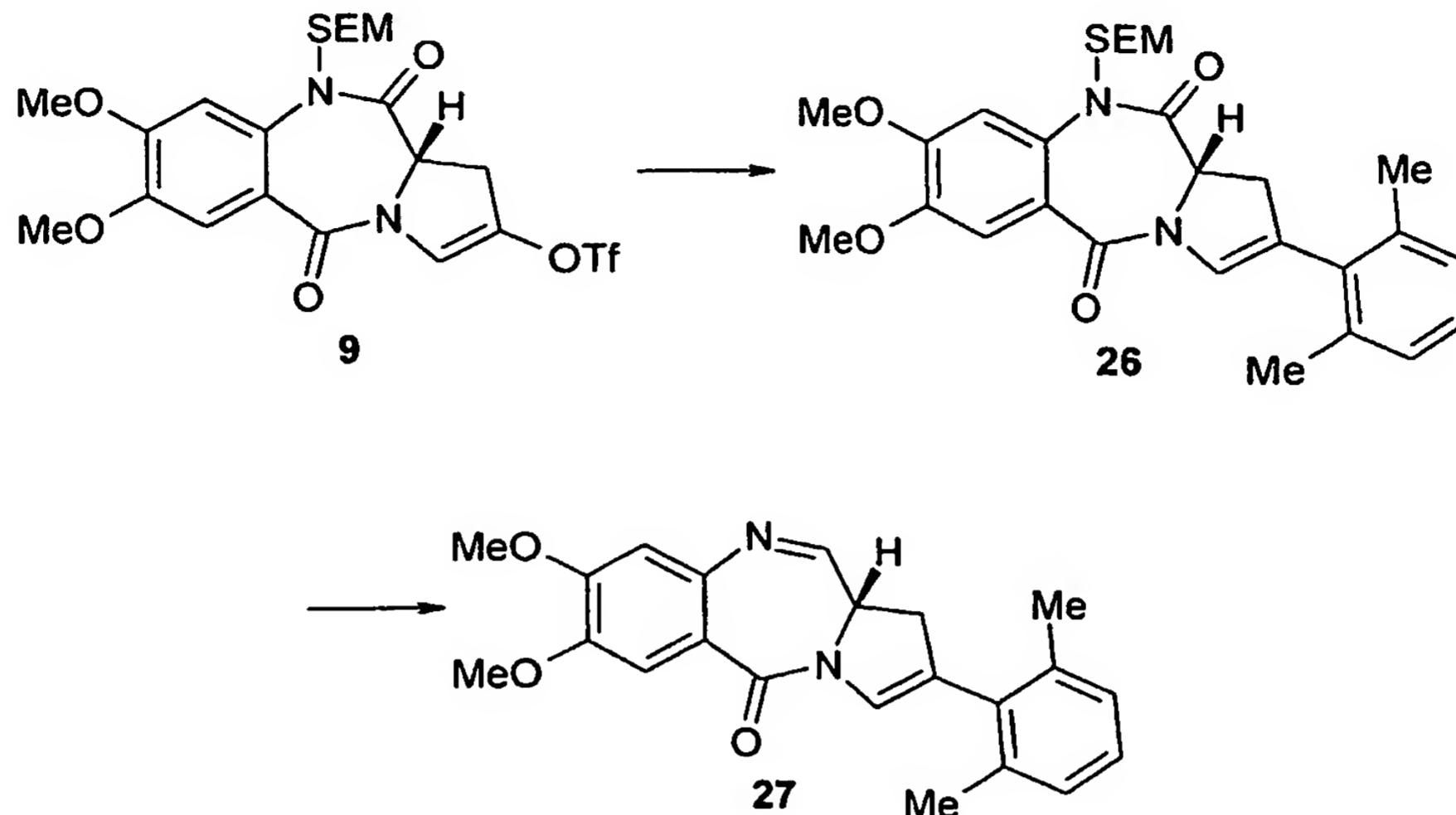
5 *(a) 2-(2,6-dimethylbenzene)-7,8-dimethoxy-10-(2-trimethylsilanyl-ethoxymethyl)-1,11a-dihydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one* **(26)**

Sodium carbonate (300 mg, 2.85 mmol), 2,6-dimethylbenzeneboronic acid (149 mg, 0.99 mmol) and

10 tetrakis(triphenylphosphine)palladium(0)(30 mg) were added to a solution of **9** (500 mg, 0.90 mmol) in ethanol/water/benzene (2/2/6 mL) and stirred at room temperature for 306.5 hours. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (20 mL) and brine (20 mL) and dried over

15 magnesium sulphate. The crude product was purified by automated flash column chromatography using a 100 - 50 % hexane in ethyl acetate gradient system as eluent to give the pure product (218 mg, 0.43 mmol, 47 % yield).

20 ^{1}H (250 MHz, CDCl$_3$) NMR : δ 7.42 (s, 1H, H-6), 7.28 (s, 1H, H-9), 7.22-7.02 (m, 3H, Ar-H x 3), 6.82 (broad s, 1H, H-3), 5.62 (d, 1H, *J* = 9.85 Hz, N-CH$_2$-OSEM x 1), 4.74 (d, 1H, *J* = 9.72 Hz, N-CH$_2$-OSEM x 1), 4.64 (dd, 1H, J = 3.08, 10.51 Hz, H-11a), 3.96 & 3.94 (2s, 6H, 7- & 8- MeO), 3.89-3.64 (m, 3H, O-CH$_2$-SEM, H-1 x 1), 3.02

25 (ddd, 1H, *J* = 2.39, 10.58, 16.89 Hz, H-1 x 1), 2.32 (s, 6H, Ar-

2,6-diMe x 6), 1.00 (t, 2H, J = 8.27 Hz, CH_2-SEM), 0.03 (s, 9H, SEM-Si$(CH_3)_3$).

(b)   (11aS)-1,11a-dihydro-7,8-dimethoxy-2-(2,4-dimethyl-benzene)-
5 *5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (27)*
Solid sodium tetraborohydride (276 mg, 7.3 mmol) was added in two portions at 0 and 24 hours to a stirred solution of **26** (208 mg, 0.41 mmol) in a mixture of anhydrous ethanol (4.1 mL) and anhydrous THF (8.2 mL). The reaction mixture was allowed to stir
10 at room temperature for 47 hours. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were evaporated *in vacuo* and the crude product was treated with ethanol (50 mL), ice water (25 mL) and silica gel (8.2 g) and left to stir at room temperature for
15 24 hours. The reaction mixture was filtered and the product extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded the crude product, which was purified using flash
20 column chromatography with a 70%-50% hexane in ethyl acetate as eluent. Pure fractions were combined and evaporated *in vacuo* to afford the pure product **27** as a yellow solid (43.3 mg, 0.12 mmol, 29 % yield).

^{1}H (250 MHZ, CDCL$_3$) NMR: δ 7.92 (d, 1H, J = 3.9 Hz, H11), 7.55 (s,
25 1H, H6), 7.34- 7.14 (m, 5H, H3, 4 x Ar-H), 6.85 (s, 1H, H9), 4.39 (dt, 1H, J = 4.7, 11.4 Hz, H11a), 3.97 & 3.95 (2s, 6H, 7- & 8- MeO), 3.78- 3.59 (m, 1H, H1), 3.47 (dd, 1H, J = 5.2, 16.2 Hz, H1), 2.49 (s, 6H, Ar-CH$_3$).

30 **Biological assays**
In vitro – clonogenic cell lines
In order to determine the activity of the compounds synthesised, solid human tumour xenografts growing subcutaneously in serial passages in thymus aplastic nude mice (Naval Medical Research
35 Institute, USA nu/nu strain) were removed under sterile condition, mechanically disaggregated and subsequently incubated

62

with an enzyme cocktail consisting of collagenase (41U/ml, Sigma), DNAse I (125 U/ml, Roche), hyaluronidase (1000 U/ml, Sigma) and dispase II(1.0 U/ml) in RPMI 1640-Medium (Life Technologies) at 37°C for 30 minutes. Cells were passed through

5   sieves of 200 µm and 50 µm mesh size and washed twice with sterile PBS-buffer. The percentage of viable cells was determined in a Neubauer-hemocytometer using trypan blue exclusion. The cells generated were as follows:

    Breast MACL MCF7

10   Renal 9442

    Lung 629L

    Melanoma MEXF 462NL

The clonogenic assay was performed in a 24-well format according

15   to a modified two-layer agar assay introduced by Hamburger, A.W. and Salmon, S.E., *Science*, **197**, 461-643 (1977). The bottom layer consisted of 0.2 ml/well of Iscove's Modified Dulbecco's Medium (supplemented with 20% fetal calf serum and 1% gentamicin) and 0.75% agar. 4×10^4 to 8×10^4 cells were added to 0.2 ml of the

20   same culture medium supplemented with 0.4 % agar and plated in 24-multiwell dishes onto the bottom layer. The test compounds were applied by continuous exposure (drug overlay) in 0.2 ml culture medium. Each dish included six control wells containing the vehicle and drug treated groups in triplicate at 6

25   concentrations. Cultures were incubated at 37°C and 7.5% CO2 in a humidified atmosphere for 8-20 days and monitored closely for colony growth using an inverted microscope. Within this period, *in vitro* tumour growth led to the formation of colonies with a diameter of >50 µm. At the time of maximum colony formation,

30   counts were performed with an automatic image analysis system (OMNICOM FAS IV, Biosys GmbH). 24 hours prior to evaluation, vital colnies were stained with a sterile aqueous solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (1 mg/ml, 100 µl/well).

35

The IC_{50} values of the test compounds were determined by plotting compound concentration versus cell viability.

The following compounds (Cooper. N., *et al.*, *Chem. Commun.*, **16**, 1764-1765 (2002) and WO 00/12508) were tested as comparisons:

C1

C2

C3
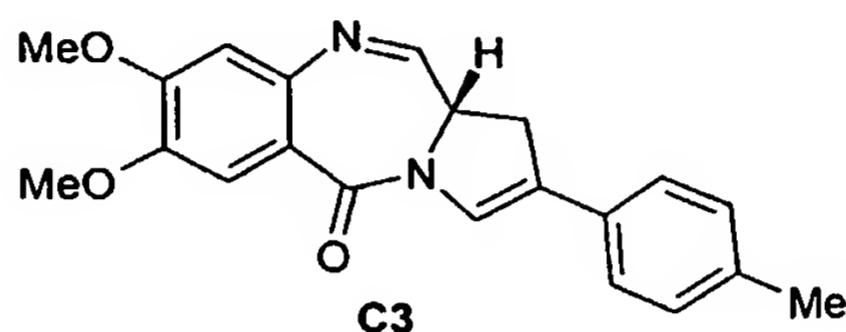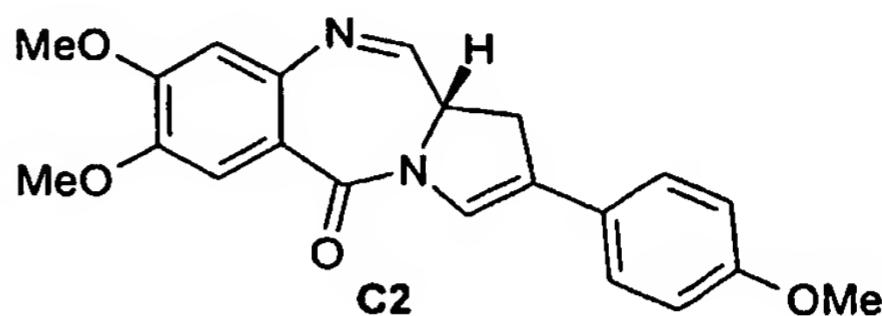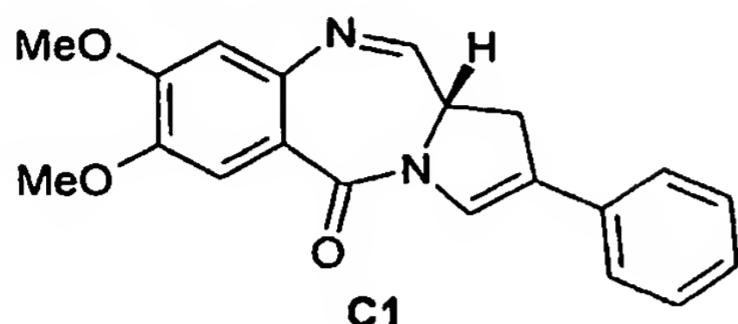
The results are as shown in the following tables.

Table 1 - Breast MACL MCF7

Compound	IC_{50} (nM)
C1	19.33
C2	1.96
C3	1.59
11	0.22
13	0.26
15	0.15
19	0.35
21	<0.1
23	0.35

Table 2 - Renal RXF 944L

Compound	IC_{50} (nM)
C1	20.50
C2	1.50
C3	0.89
17	0.17
21	<0.1

64

Table 3 - Lung 629L

Compound	IC$_{50}$ (nM)
C1	44.50
C2	5.00
C3	8.00
19	1.00
21	0.36

Table 4 - Melanoma MEXF 462NL

Compound	IC$_{50}$ (nM)
C1	40.00
C2	2.85
C3	2.00
21	<0.1

5 NCI *In Vitro* Cytotoxicity Studies

The National Cancer Institute (NCI), Bethesda, Maryland, USA has available an *in vitro* cytotoxicity screen which consists of approximately 60 human tumour cell lines against which compounds are tested at a minimum of five concentrations each differing 10-
10 fold. A 48 hour continuous exposure protocol is used, where cell viability or growth is estimated with an SRB protein assay.

Method
The test compounds were evaluated against approximately 60 human
15 tumour cell lines. The NCI screening procedures were described in detail by Monks and co-workers (Monks, A *et al.*, Journal of the National Cancer Institute, 1991, 83, 757). Briefly, cell suspensions were diluted according to the particular cell type and the expected target cell density (5000-40,000 cells per well
20 based on cell growth characteristics), and added by pipette (100 μL) into 96-well microtitre plates. The cells were allowed a preincubation period of 24 hours at 37°C for stabilisation. Dilutions at twice the intended test concentration were added at time zero in 100 μL aliquots to the wells. The test compounds
25 were evaluated at five 10-fold dilutions (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}

and 10^{-8} µM). The test compounds were incubated for 48 hours in 5% CO_2 atmosphere and 100% humidity. The cells were then assayed using the sulphorhodamine B assay. A plate reader was used to read the optical densities and a microcomputer processed the

5   readings into GI_{50} values (in Moles), which is the dosage required to limit cell growth to 50%.

The results of these studies can be summarized by averaging log GI_{50} across all the cell lines tested to derive a MG_MID value,

10   which shows that compound 25 (MG_MID = -7.18) is more effective than compound 23 (MG_MID = -6.34).

NCI Hollow Fibre Assay for Preliminary *In Vivo* Testing
The Biological testing Branch of the Developmental Therapeutics

15   Program of the NCI has adopted a preliminary *in vivo* screening tool for assessing the potential anticancer activity of compounds identified by the large scale *in vitro* cell screen. For these assays, human tumour cells are cultivated in polyvinylidene (PVDF) hollow fibres, and a sample of each cell line is implanted

20   into each of two physiologic compartments (intraperitoneal and subcutaneaous) in mice. Each test mouse received a total of 6 fibres (3 intraperitoneally and 3 subcutaneously) representing 3 distinct cancer cell lines. These mice are treated with potential antitumour compounds at each of 2 test doses by the .

25   intraperitoneal route using a QD x 4 treatment schedule. Vehicle controls consist of 6 mice receiving the compound diluent only. The fibre cultures are collected on the day following the last day of treatment. To assess anticancer effects, the viable cell mass is determined for each of the cell lines using a formazyn

30   dye (MTT) conversion assay. From this, the %T/C can be calculated using the average optical density of compound treated samples divided by the average optical; density of the vehicle controls. In addition, the net increase in cell mass can be determined for each sample, as a sample of fibre cultures are

35   assessed for viable cell mass on the day of implantation into mice. Thus, the cytostatic and cytocidal capacities of the test

compound can be assessed.

Generally, each compound is tested against a minimum of 12 human
cancer cell lines. This represents a total of 4 experiments
5   since each experiment contains 3 cell lines. The data are
reported as %T/C for each of the 2 compound doses against each of
the cell lines with separate values calculated for the
intraperitoneal and subcutaneous samples.

10 Compounds are selected for further *in vivo* testing in standard
subcutaneous xenograft models on the basis of several hollow
fibre assay criteria. These include: (1) a %T/C of 50 or less in
10 of the 48 possible test combinations (12 cell lines X 2 sites
X 2 compound doses); (2) activity at a distance (intraperitoneal
15 drug/subcutaneous culture) in a minimum of 4 of the 24 possible
combinations; and/or (3) a net cell kill of 1 or more of the cell
lines in either implant site. To simplify evaluation, a points
system has been adopted which allows rapid evaluation of the
activity of a given compound. For this, a value of 2 is assigned
20 for each compound dose which results in a 50% or greater
reduction in viable cell mass. The intraperitoneal and
subcutaneous samples are scored separately so that criteria (1)
and (2) can be evaluated. Compounds with a combined IP + SC
score of 20, a SC score of 8 or a net cell kill of one or more
25 cell lines are referred for xenograft testing. This comparison
indicated that there was a very low probability of missing an
active compound if the hollow fibre assay was used as the initial
in vivo screening tool. In addition to these criteria, other
factors (e.g. unique structure, mechanism of action) may result
30 in referral of a compound for xenograft testing without the
compound meeting these criteria.

Certain compounds of the invention were tested in variations of
these assays, to assess their activity against renal and melanoma
35 cell lines. In the melanoma assay, only 6 cell lines are used,
and therefore compounds with a combined IP + SC score of 10 or a
SC score of 4 are referred for xenograft testing. In the renal

assay, only three cell lines are used and therefore compounds with a combined IP + SC score of 5 or a SC score of 2 are referred for xenograft testing.

5   The results of this assay are presented below in tables 5 and 6, including results for some of the compounds disclosed in Cooper. N., *et al.*, *Chem. Commun.*, **16**, 1764-1765 (2002) and WO 00/12508:

Compound	IP	SC	IP + SC
C1	0	0	0
C3	2	0	2
13	4	2	6
27	4	4	8

Table 5 - Renal

10

Compound	IP	SC	IP + SC
C1	4	0	4
C3	0	2	2
13	2	4	6
19	4	4	8
21	10	0	10

Table 6 - Melanoma

On the basis of these results, compounds 13 and 27 have been
15   selected to be tested in the next stage of the NCI program (xenografts) on renal cancers, whilst compounds 12 and 21 have been selected to be tested on melanomas.